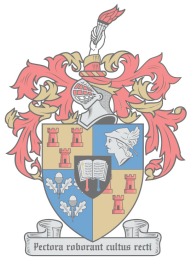
Strategic nitrogen fertilisation of kikuyu-based pastures over-sown with legumes

by

Bernhard Jordaan



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Declaration

By submitting this thesis electronically, I declare that the entirety of the work contained therein is my own, original work, that I am the sole author thereof (save to the extent explicitly otherwise stated), that reproduction and publication thereof by Stellenbosch University will not infringe any third party rights and that I have not previously in its entirety or in part submitted it for obtaining any qualification.

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Abstract

Kikuyu (*Pennisetum clandestinum*)-ryegrass (*Lolium* spp.) pastures for dairy production in the southern Cape of South Africa often receive nitrogen (N) fertilisation rates in excess of 500 kg ha^{-1} yr^{-1}. To reduce the high N fertilisation inputs of the region, legumes have been incorporated into these grass pasture systems. Legumes fix atmospheric N and can improve nutritive value of the pasture. However, research concerning N fertilisation guidelines of grass-legume pastures in the southern Cape is limited. As result of this limited research, producers may be applying varying N rates to grass-legume pastures, which may result in these systems being environmentally and economically unsustainable. The aim of this study was to determine the effect of N fertilisation regimes on soil and pasture parameters of a kikuyu-perennial ryegrass (*L. perenne*)-clover (*Trifolium* spp.) (KPrCl) and a kikuyu-lucerne (*Medicago sativa*) (KL) pastures under grazing and irrigation. Five N fertilisation regimes were used as treatments and were applied after each grazing event [0 (N0), 20 (N20), 40 (N40), 60 kg N ha^{-1} (N60) and a variable N application (N_{var})]. Nitrate concentration measured from wetting front detectors as well the distribution of total soil N between the depths 0-100, 100-200 and 200-300 mm indicated that a significant amount of leaching may have occurred throughout the study. However, leaching was constant for all treatments on both pasture types. Furthermore N treatments had an minimal influence on total soil N, potential mineralisable N and urease activity. The only soil parameter that showed a significant reaction to N fertilisation regimes was total inorganic soil N. For both pasture types, total inorganic soil N increased under the N60 fertilisation regime. Seasonal herbage yield for the KPrCl type was only affected by N treatment in the spring (year one), where N60 produced a higher yield than N0 and N_{var}. Treatment had no effect on total annual yield of KPrCl pastures. For the KL type, treatment N60 produced the highest or similar to the highest yield in the spring, summer and autumn. The annual yield under treatment N20, N40 and N60 were higher than N0 and N_{var}. Botanical composition for both pasture types were affected differently by N treatments. For the KPrCl type, kikuyu was positively related to N fertilisation rates in autumn, whilst ryegrass was positively related to N rates in the winter and spring. For most seasons the clover component responded negatively to N fertilisation. For the KL pasture, kikuyu was not affected by N treatments. Lucerne only responded to N fertilisation in the summer, during which N40 had the highest lucerne component. The crude protein content of the KPrCl pasture type was highest or similar to the highest under treatment N60 for all months, whilst crude protein for the KL type was highest under N60 from February to June.

Uittreksel

Dit is n algemene praktyk vir suiwelprodusente in die Suid-Kaap streek van Suid-Afrika om kikoejoe (*Pennisetum clandestinum*)-raaigras (*Lolium* spp.)-weidings teen stikstof (N) peile van meer as 500 kg N ha^{-1} $jaar^{-1}$ te bemes. Om hierdie hoë N-peile te verlaag, word peuleplante saam met grasweidings gevestig. Peulplante het die vermoë om atmosferiese N na n plant beskikbare-vorm om te skakel. Navorsingsinligting op N-bemsetingsriglyne van gras-peulplant weidings in die Suid-Kaap is egter beperk. As gevolg van die beperkte navorsingsinligting, word dit verwag dat produsente wisselende N-peile toedien. Dit kan lei na ekonomiese en omgewingsnagevolge. Daarom is die doel van hierdie studie om die effek van N-bememstingspeile op grond- en weidingsparameters van kikoejoe-meerjarige raaigras (*L. perenne*)-klawer (*Trifolium* spp.) (KPrCl) en kikoejoe-lusern (*Medicago sativa*) (KL)-weidings onder beweiding en besproeiing, te bepaal, Vyf bemestingspeile was getoets as behandelinge en was toegedien na beweiding [(N0), 20 (N20), 40 (N40), 60 kg N ha^{-1} (N60) en ’n wisselende N-peil (N_{var})]. Nitraatkonsentrasie wat vanaf benattingsfrontaanwysers bepaal is, asook die verspreiding van totale grond-N tussen dieptes 0-100, 100-200 and 200-300 mm, het daarop gedui dat loging plaasgevind het, maar dat loging nie deur N-bemesting beïnvloed was nie. Totale grond-N, potensieël mineraliseerbare N en urease aktiwiteit was minimaal beïnvloed deur N-bemestingspeile. Die enigste grondparameter wat reaksie tot N bemesting getoon het, was totale anorganiese grond-N. Vir beide weidingstipes het totale anorganiese grond-N onder behandeling N60 deur die verloop van die studie verhoog. Seisoenale opbrengs van die KPrCl weidingstipe was net in die lente van jaar een deur N bemestingspeile beïnvloed waar die opbrengs van behandeling N60 hoër as N0 en N_{var} was. Behandeling het geen effek op die jaarlikse opbrengs gehad nie. Vir die KL-weidingstipe was opbrengs in die lente, somer en herfs van behandeling N60 die hoogste of gelykstaande aan die hoogste. Die jaarlikse opbrengs vir N20, N40 en N60 was vir die eerste jaar van die studie hoër as N0 en N_{var}. Botaniese samestelling van beide weidingstipes was deur N-bemesting beïnvloed. Vir die KPrCl-tipe was die kikoejoekomponent positief met N-bemesting in die herfs gekorreleer, terwyl raaigras positief met N bemesting tydens die winter en lente gekorreleerd was. Vir meeste van die seisoene was die klawerkomponent negatief met N bemesting gekorreleerd. Met betrekking tot die KL-tipe, het die kikoejoekomponent nie op N-peile nie gereageer. Die lusernkomponent het net in die somer op N bemesting gereageer. In die somer was die lusernkomponent van N40 hoër as die ander behandelings. Die ru-proteininhoud van die KPrCl-tipe was onder behandeling N60 die hoogste of naaste aan die hoogste, terwyl ru-proteininhoud van die KL-tipe die hoogste vir N60 vanaf Februarie tot Junie was.

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Abbreviations

ANOVA	Analysis of variance
BNF	Biological Nitrogen fixation
^{o}C	Degrees Celsius
C	Carbon
c.	Circa (about)
CEC	Cation exchange capacity
cv.	Cultivar
DM	Dry matter
E	East
g	gram
H	Hydrogen
ha	hectare
KCl	Potassium Chloride
Kg	Kilogram
KL	Kikuyu-lucerne
kPa	Kilopascal
KPrCl	Kikuyu-perennial ryegrass-clover
L	liter
LAN	Limestone-ammonium nitrate
m	meter
meq	milliequivalents
mg	milligrams
mm	millimeter
MPa	Megapascal
N	Nitrogen
NH_4^+	Ammonium
NO_2^-	Nitrite
NO_3^-	Nitrate
N_{var}	Variable nitrogen treatment
N0	0 kg N ha^{-1} treatment
N20	20 kg N ha^{-1} treatment
N40	40 kg N ha^{-1} treatment
N60	60 kg N ha^{-1} treatment
O	Oxygen
P	Probability value
PMN	Potentially mineralisable nitrogen

S	South
SOM	Soil organic matter
spp.	Species
t	ton
WFD	Wetting front detector
yr	Year

Table of contents

Abstract ..... **i**

Uittreksel ..... **ii**

Acknowledgements ..... **iv**

Abbreviations ..... **v**

Table of contents ..... **vii**

List of Figures ..... **xi**

List of Tables ..... **xv**

CHAPTER 1 ..... **1**

Introduction 1

1.1 Introduction 1

1.2 Problem identification 2

1.3 Aims and objectives 3

1.4 Structure of the thesis 4

1.5 References 5

CHAPTER 2 ..... **8**

Literature review 8

2.1 Nitrogen 8

2.1.1 Introduction 8

2.1.2 Nitrogen cycle 8

2.1.3 Biological N fixation 15

2.2 Kikuyu (Pennisetum clandestinum) 18

2.2.1 General 18

2.2.2 Growth requirements 18

2.2.3 Pasture production 19

2.3 Perennial ryegrass (Lolium perenne) 19

2.3.1 General 19

2.3.2 Growth requirements: 19

2.4 Lucerne (Medicago sativa) 21

2.4.1 General 21

2.4.2 Growth requirements 22
2.5 White clover (Trifolium repens L.) 22
2.5.1 General 22
2.5.2 Growth requirements 23
2.6 Red clover (Trifolium pratense) 24
2.6.1 General 24
2.6.2 Growth requirements 24
2.7 Grass-legume pastures 25
2.7.1 Interaction with nitrogen 25
2.7.2 Pasture production 25
2.7.3 Nitrogen uptake 26
2.8 References 28
CHAPTER 3 37
Variable nitrogen fertilisation and soil nitrogen dynamics of kikuyu-based pastures over-sown with perennial ryegrass and legumes 37
3.1 Introduction 37
3.2 Materials and methods 38
3.2.1 Experimental site 38
3.2.2 Experimental design and treatments 38
3.2.3 Seed treatment and establishment 39
3.2.4 Irrigation scheduling 40
3.2.5 Grazing and pasture allocation 40
3.2.6 Soil sampling and analyses 41
3.2.7 Statistical analysis 42
3.3 Results and discussions 42
3.3.1 Kikuyu-perennial ryegrass-clover soil nitrate 42
3.3.2 Kikuyu-perennial ryegrass-clover total soil nitrogen 43
3.3.3 Kikuyu-perennial ryegrass-clover total inorganic soil nitrogen 45
3.3.4 Kikuyu-perennial ryegrass-clover potential mineralisable soil nitrogen 46
3.3.5 Kikuyu-perennial ryegrass-clover soil urease activity 47

3.3.6 Kikuyu-lucerne soil nitrate 48
3.3.7 Kikuyu-lucerne total soil nitrogen 49
3.3.8 Kikuyu-lucerne total inorganic soil nitrogen 51
3.3.9 Kikuyu-lucerne soil urease activity 52
3.4 Conclusions and recommendations 53
3.5 References 55
CHAPTER 4 58
Nitrogen fertilisation effects on herbage yield, quality and botanical composition of kikuyu-based pastures over-sown with ryegrass and legumes 58
4.1 Introduction 58
4.2 Materials and methods 59
4.2.1 Experimental site 59
4.2.2 Experimental treatments and layout 60
4.2.3 Seed treatment and establishment 62
4.2.4 Grazing management 63
4.2.5 Irrigation management 63
4.2.6 Herbage yield and herbage protein 63
4.2.7 Botanical composition 64
4.2.8 Statistical analyses 64
4.3 Results and discussions 64
4.3.1 Kikuyu-perennial ryegrass-clover herbage production 64
4.3.2 Kikuyu-perennial ryegrass-clover botanical composition 67
4.3.3 Kikuyu-perennial ryegrass-clover crude protein 72
4.3.4 Kikuyu-lucerne herbage production 73
4.3.5 Kikuyu-lucerne botanical composition 75
4.3.6 Kikuyu-lucerne crude protein 79
4.4 Conclusion and recommendations 81
4.5 References 82
CHAPTER 5 86
Summary and recommendations 86

5.1 Synopsis 86

5.2 General conclusion 88

5.3 Recommendations for further research 89

5.4 References 91

List of Figures

Figure 2.1.1. Simplified version of the N cycle (Adopted from Hofman and Van Cleemput 2004).... 9

Figure 2.1.2. The relationship between pH and distribution of NH_3 and NH_4^+ (adopted from Court et al. 1964)..... 12

Figure 2.1.3. The relationship between microbial activity and water filled pore space (adopted from Linn and Doran 1984). 14

Figure 2.3.1. The seasonal herbage production of perennial ryegrass and kikuyu-perennial ryegrass pastures (adapted from Van der Colf et al. 2015 and Van der Colf et al. 2016). 21

Figure 2.7.1. The seasonal DM production of kikuyu-perennial ryegrass-clover (KPrCl) and kikuyu-lucerne (KL) pastures (Adapted from Fourie 2015; Van der Colf 2016). 26

Figure 3.2.1. The average soil water content (gravimetric) for the kikuyu-perennial ryegrass-clover pasture site on the day of sampling. The soil water content is reported for depths of 0-100, 100-200 and 200-300 mm. 41

Figure 3.2.2. The average soil water content (gravimetric) for the kikuyu-lucerne pasture site on the day of sampling. The soil water content is reported for depths of 0-100, 100-200 and 200-300 mm..... 42

Figure 3.3.1. The average nitrate concentration measured by wetting front detectors at depths of 150 and 300mm respectively. Error bars indicate standard error where more than one observation were recorded. The arrow indicates the average N applied to the four N_{var} plots (6.25 kg N ha^{-1}). 43

Figure 3.3.2. Total soil nitrogen to a depth of 300 mm per season under various fertiliser regimes. Fertiliser regimes: N0, N20, N40, N60 = 0, 20, 40, 60 kg N ha^{-1} applied after grazing; N_{var} = variable nitrogen fertilisation. Error bars indicate standard error. No common letter above bars denotes a significant difference ($P<0.05$). 44

Figure 3.3.3. The percentage contribution of total soil N content at depths of 0-100mm, 100-200mm and 200-300mm. Treatments were compared within season per depth. N0, N20, N40, N60 = 0, 20, 40, 60 kg N ha^{-1}; N_{var} = variable nitrogen fertilisation. 45

Figure 3.3.4. Total inorganic soil nitrogen to a depth of 100 mm per month under various fertiliser regimes. Fertiliser regimes: N0, N20, N40, N60 = 0, 20, 40, 60 kg N ha^{-1} applied after grazing; N_{var} = variable nitrogen fertilisation. Error bars indicate standard error. No common letter above bars denotes a significant difference ($P<0.05$). 46

Figure 3.3.5. Urease activity to a depth of 100 mm under various fertiliser regimes. Fertiliser regimes: N0, N20, N40, N60 = 0, 20, 40, 60 kg N ha^{-1} applied after grazing; N_{var} = variable

nitrogen fertilisation. Error bars indicate standard error. No common letter above bars denotes a significant difference (P<0.05). 47

Figure 3.3.6. The average nitrate concentration as measured by wetting front detectors at depths of 150 and 300 mm respectively. Error bars indicate standard error. Arrow indicates the average N applied to the four N_{var} plots (25 kg N ha^{-1}). 48

Figure 3.3.7. Total soil nitrogen to a depth of 300 mm per season under various fertiliser regimes. Fertiliser regimes: N0, N20, N40, N60 = 0, 20, 40, 60 kg N ha^{-1} applied after grazing; N_{var} = variable nitrogen fertilisation. Error bars indicate standard error. No common letter above bars denotes a significant difference (P<0.05). 50

Figure 3.3.8. The percentage contribution of total N content at depths of 0-100mm, 100-200mm and 200-300mm. Treatments were compared within season per depth. N0, N20, N40, N60 = 0, 20, 40, 60 kg N ha^{-1}; N_{var} = variable nitrogen fertilisation. 51

Figure 3.3.9. Total inorganic soil nitrogen to a depth of 100 mm per month under various fertiliser regimes. Fertiliser regimes: N0, N20, N40, N60 = 0, 20, 40, 60 kg N ha^{-1} applied after grazing; N_{var} = variable nitrogen fertilisation. Error bars indicate standard error. No common letter above bars denotes a significant difference (P<0.05). 51

Figure 3.3.10. Urease activity to a depth of 100 mm under various fertiliser regimes. Fertiliser regimes: N0, N20, N40, N60 = 0, 20, 40, 60 kg N ha^{-1} applied after grazing; N_{var} = variable nitrogen fertilisation. Error bars indicate standard error. No common letter above bars denotes a significant difference (P<0.05). 53

Figure 4.3.1. The total annual herbage yield (kg DM ha^{-1} yr^{-1}) of a kikuyu-perennial ryegrass-clover pasture, during year one, in response to nitrogen fertilisation treatments applied after grazing. N0, N20, N40, N60 = 0, 20, 40, 60 kg N ha^{-1}; N_{var} =variable nitrogen fertilisation. No common letter above bars denotes a significant difference (P<0.05). 67

Figure 4.3.2. Seasonal changes in kikuyu's contribution to botanical composition in response to nitrogen fertilisation treatments that were applied after grazing. N0, N20, N40, N60 = 0, 20, 40, 60 kg N ha^{-1}; N_{var} = variable nitrogen fertilisation. Error bars indicate standard error. No common letter above bars denotes a significant difference (P<0.05) 69

Figure 4.3.3. Seasonal changes in ryegrass's contribution to botanical composition in response to nitrogen fertilisation treatments that were applied after grazing. N0, N20, N40, N60 = 0, 20, 40, 60 kg N ha^{-1}; N_{var} = variable nitrogen fertilisation. Error bars indicate standard error. No common letter above bars denotes a significant difference (P<0.05). 69

Figure 4.3.4. Seasonal changes in clover's contribution to botanical composition in response to nitrogen fertilisation treatments that were applied after grazing. N0, N20, N40, N60 = 0, 20,

40, 60 kg N ha^{-1}; N_{var} = variable nitrogen fertilisation. Error bars indicate standard error. No common letter above bars denotes a significant difference (P<0.05). 70

Figure 4.3.5. Seasonal changes in weeds' contribution to botanical composition in response to nitrogen fertilisation treatments that were applied after grazing. N0, N20, N40, N60 = 0, 20, 40, 60 kg N ha^{-1}; N_{var} = variable nitrogen fertilisation. Error bars indicate standard error. No common letter above bars denotes a significant difference (P<0.05). 70

Figure 4.3.6. Seasonal changes in other grass's contribution to botanical composition in response to nitrogen fertilisation treatments that were applied after grazing. N0, N20, N40, N60 = 0, 20, 40, 60 kg N ha^{-1}; N_{var} = variable nitrogen fertilisation. Error bars indicate standard error. No common letter above bars denotes a significant difference (P<0.05). 71

Figure 4.3.7. Non–metric multidimensional scaling ordination of the effect of N fertilisation treatments, applied after grazing, on pasture species composition over seasons during year one. N0, N20, N40, N60 = 0, 20, 40, 60 kg N ha^{-1}; N_{var} = variable N application. Axis 1 and 2. R^2 = 0.06, P = 0.023. 72

Figure 4.3.8. Monthly changes in herbage crude protein content in response to nitrogen fertilisation treatments that were applied after grazing. N0, N20, N40, N60 = 0, 20, 40, 60 kg N ha^{-1}; N_{var} = variable nitrogen fertilisation. Treatments were compared within months. Error bars indicate standard error. No common letter above bars denotes a significant difference within months (P<0.05). 73

Figure 4.3.9. The total annual herbage yield (kg DM ha^{-1} yr^{-1}) of a kikuyu-lucerne pasture, during year one, in response to nitrogen fertilisation treatments applied after grazing. N0, N20, N40, N60 = 0, 20, 40, 60 kg N ha^{-1}; N_{var} = variable nitrogen fertilisation. Error bars indicate standard error. No common letter above bars denotes a significant difference (P<0.05). 75

Figure 4.3.10. Seasonal changes in kikuyu's contribution to botanical contribution in response to nitrogen fertilisation treatments that were applied after grazing. N0, N20, N40, N60 = 0, 20, 40, 60 kg N ha^{-1}; N_{var} = variable nitrogen fertilisation. Error bars indicate standard error. No common letter above bars denotes a significant difference (P<0.05). 76

Figure 4.3.11. Seasonal changes in lucerne's contribution to botanical contribution in response to nitrogen fertilisation treatments that were applied after grazing. N0, N20, N40, N60 = 0, 20, 40, 60 kg N ha^{-1}; N_{var} = variable nitrogen fertilisation. Error bars indicate standard error. No common letter above bars denotes a significant difference (P<0.05). 76

Figure 4.3.12. Seasonal changes in other grass's contribution to botanical contribution in response to nitrogen fertilisation treatments that were applied after grazing. N0, N20, N40, N60 = 0, 20, 40, 60 kg N ha^{-1}; N_{var} =variable nitrogen fertilisation. Error bars indicate standard error. No common letter above bars denotes a significant difference (P<0.05). 77

Figure 4.3.13. Seasonal changes in other legumes' contribution to botanical contribution in response to nitrogen fertilisation treatments that were applied after grazing. N0, N20, N40, N60 = 0, 20, 40, 60 kg N ha^{-1}; N_{var} =variable nitrogen fertilisation. Error bars indicate standard error. No common letter above bars denotes a significant difference (P<0.05)..... 78

Figure 4.3.14. Seasonal changes in weeds' contribution to botanical contribution in response to nitrogen fertilisation treatments that were applied after grazing. N0, N20, N40, N60 = 0, 20, 40, 60 kg N ha^{-1}; N_{var} =variable nitrogen fertilisation. Error bars indicate standard error. No common letter above bars denotes a significant difference (P<0.05). 79

Figure 4.3.15. Non–metric multidimensional scaling ordination of the effect of N fertilisation treatments (applied after grazing) on pasture species composition over seasons, during year one. N0, N20, N40, N60 = 0, 20, 40, 60 kg N ha^{-1}; N_{var} = variable N application. Axis 1 and 2. R^2 0.07, P = 0.015..... 80

Figure 4.3.16. Monthly changes in herbage crude protein content in response to nitrogen fertilisation treatments that were applied after grazing. N0, N20, N40, N60 = 0, 20, 40, 60 kg N ha^{-1}; N_{var} = variable nitrogen fertilisation. Treatments were compared within months. Error bars indicate standard error. No common letter above bars denotes a significant difference within months (P<0.05). 81

List of Tables

Table 3.2.1. The description of soil- horizons and texture at different depths, found at experimental site. 38

Table 3.2.2. The pesticide type, active ingredient, commercial product and dosage rate used to treat lucerne and clover seeds before planting. 40

Table 3.3.1. Total soil nitrogen (mg kg^{-1}) to a depth of 100 mm, per month under various fertiliser regimes. Fertiliser regimes: N0, N20, N40, N60 = 0, 20, 40, 60 kg N ha^{-1} applied after grazing; N_{var} = variable nitrogen fertilisation. 44

Table 3.3.2. Potential mineralisable nitrogen (kg N ha^{-1} grazing $cycle^{-1}$) to a depth of 100 mm per month under various fertiliser regimes. Fertiliser regimes: N0, N20, N40, N60 = 0, 20, 40, 60 kg N ha^{-1} applied after grazing; N_{var} = variable nitrogen fertilisation. 47

Table 3.3.3. Total soil nitrogen (mg kg^{-1}) to a depth of 100 mm per month under various fertiliser regimes. Fertiliser regimes: N0, N20, N40, N60 = 0, 20, 40, 60 kg N ha^{-1} applied after grazing; N_{var} = variable nitrogen fertilisation. 49

Table 3.3.4. Potential mineralisable nitrogen (kg N ha^{-1} grazing $cycle^{-1}$) to a depth of 100 mm per month under various fertiliser regimes. Fertiliser regimes: N0, N20, N40, N60 = 0, 20, 40, 60 kg N ha^{-1} applied after grazing; N_{var} = variable nitrogen fertilisation. 52

Table 4.2.1. Pasture type, over-sown species and cultivars used in this study. 59

Table 4.2.2. Nitrogen input for fixed nitrogen fertilisation treatments. 60

Table 4.2.3. Actual amounts of N fertilised, for the kikuyu-perennial ryegrass-clover pasture type, per season for each treatment in kg N ha^{-1} 61

Table 4.2.4. Actual amounts of N fertilised, for the kikuyu-lucerne pasture type, per season for each treatment in kg N ha^{-1}. 61

Table 4.2.5. Guidelines used to determine N_{var} rate from nitrate concentration measured. 62

Table 4.2.6. The pesticide type, active ingredient and dosage rate used to treat leguminous seeds before planting. 63

Table 4.3.1. The total monthly herbage yield (kg DM ha^{-1}) of a kikuyu-perennial ryegrass-clover pasture in response to nitrogen fertilisation treatments applied after grazing. N0, N20, N40, N60 = 0, 20, 40, 60 kg N ha-1; N_{var} =variable nitrogen fertilisation. Treatments were compared within months. No common denotes a significant difference ($P<0.05$) 66

Table 4.3.2. The total seasonal herbage yield (kg DM ha^{-1}) of a kikuyu-perennial ryegrass-clover pasture in response to nitrogen fertilisation treatments applied after each grazing. N0, N20, N40, N60=0, 20, 40, 60 kg N ha^{-1}; N_{var}=variable nitrogen fertilisation. 66

Table 4.3.3. The total monthly yield (kg DM ha^{-1} yr^{-1}) of a kikuyu-lucerne pasture in response to nitrogen fertilisation treatments applied after grazing. N0, N20, N40, N60 = 0, 20, 40, 60 kg N ha-1; Nvar =variable nitrogen fertilisation. Treatments were compared within months. No common letter denotes a significant difference (P<0.05). 74

Table 4.3.4. The total seasonal herbage yield (kg DM ha^{-1}) of a kikuyu-lucerne pasture in response to nitrogen fertilisation treatments applied after each grazing. N0, N20, N40, N60 = 0, 20, 40, 60 kg N ha^{-1}; N_{var}=variable nitrogen fertilisation. 75

Table 5.2.1. Comparison between a conventional N fertilisation regime and a variable N fertilisation regime for a kikuyu-perennial ryegrass-clover pasture. 89

Table 5.2.2. Comparison between a conventional N fertilisation regime and a variable N fertilisation regime for a kikuyu-lucerne pasture. 89

CHAPTER 1

Introduction

1.1 Introduction

The dairy production region on the southern seaboard of South Africa stretches between Swellendam in the Western Cape Province and the Gamtoos valley in the Eastern Cape Province (Swanepoel *et al.* 2015a). The dairy farming systems in this region are predominately based on cultivated pastures (Botha *et al.* 2008a). Irrigated kikuyu (*Pennisetum clandestinum*) serves as one of the dominant pasture bases for the summer and autumn months in this region (Botha *et al.* 2008a). Kikuyu, a C_4 grass, needs air temperatures of 16 to 21^{0}C and c. 850 to 1300 mm rain to grow optimally (Marias 2001). It therefore thrives under irrigation in the southern Cape, where average summer temperatures vary between 18 and 25^{0}C, and rainfall is c. 730 mm annually (ARC-ISCW 2017). From summer to autumn, the average dry matter (DM) production of kikuyu can reach 70 kg DM ha^{-1} day^{-1} (Botha *et al.* 2008a; Swanepoel *et al.* 2014). It can therefore support high stocking rates (Mears 1970; Botha *et al.* 2008b). Moreover, it is a densely stoloniferous and rhizomateous grass, which enables it to withstand heavy grazing (Marias 2001). However, pasture quality is low compared to temperate grass species (Marais 2001; Botha *et al.* 2008b; Garcia *et al.* 2014) and kikuyu reaches dormancy in winter and production declines. Kikuyu's production can decrease from summer to winter by up to 70% (Swanepoel *et al.* 2014). On the southern seaboard, temperate pasture species are generally over-sown into kikuyu to maintain fodder flow from winter to spring due to their complementary growth patterns to that of kikuyu (Minson *et al.* 1993; Van der Colf 2015a). Ryegrass (*Lolium* spp.) is a common species used to over-sow kikuyu in this region. Ryegrass has the ability to produce optimally from winter to spring and contribute to high herbage yield and quality (Botha *et al.* 2008a; Botha *et al*. 2008b; Van der Colf 2015a; Van der Colf *et al.* 2015b). Botha *et al.* (2008a) determined that the annual yield of a kikuyu based pasture increased from 13.8 to 19.3 kg DM ha^{-1} yr^{-1} after annual ryegrass (*L. multiflorum*) was over-sown in autumn.

The intensive farming practises that are associated with kikuyu-ryegrass pastures can, however, exhaust and/or pollute natural resources (Hardarson and Atkins 2003; Swanepoel *et al.* 2015a; Swanepoel *et al.* 2015b). In particular, high nitrogen (N) fertilisation rates (300 to 500 kg N ha^{-1} yr^{-1}) have led producers to include legumes into these grass pasture systems (Swanepoel *et al.* 2015b). Theoretically, over-sowing of legumes in mixtures with ryegrass, or as a replacement for ryegrass, can reduce this environmental pollution resulting from high nitrogen fertiliser application rates (Cransberg and McFarlane 1994; Ledgard 2001). Legumes associate with *Rhizobium* bacteria to form root nodules that fix atmospheric N into a plant-available source of N (Ledgard and Steele

1992; Hardarson and Atkins 2003). Legumes thus have the potential to decrease inorganic N requirements of cultivated pastures (Ledgard and Steele 1992; Ledgard *et al.* 2001). Leguminous herbage also has a higher quality and better digestibility, palatability and nutritional value than most temperate grass species (Botha *et al.* 2008b). Legumes, such as clovers (*Trifolium* spp.), are suitable for over-sowing grass-based pastures. For the southern Cape region, it has been determined that kikuyu-clover pastures produce annual herbage yields that are comparable to kikuyu-ryegrass (Botha *et al.* 2008a; Botha *et al.* 2008b). Clover is, however, not the only leguminous species cultivated in the southern Cape. A portion of dryland pastures are generally based on lucerne *(Medicago sativa)*, for both sheep and dairy production (van Heerden and Botha 1995). Although lucerne is well adapted to dryland conditions, due to a deep taproot system (Gault *et al.* 1995), it is occasionally cultivated under irrigation in dairy production systems in the region where the soil is physically and chemically suitable. Under irrigation, lucerne has the ability to produce 10 to 13 t DM ha^{-1} per annum and fix up to 370 kg N ha^{-1} yr^{-1} (Gault *et al.* 1995). Van Heerden and Botha (1995) compared lucerne to clover-based pastures under dry land conditions in the southern Cape and reported that the lucerne-based pastures continuously maintained a higher stocking rate and herbage yield than the clover-based pastures. Therefore, the incorporation of legumes into grass pastures can potentially result in improved quality, lower N fertilisation rates and still maintain yields similar to grass pastures.

1.2 Problem identification

The world population in the 1960s stood at roughly three billion people. Since then it has more than doubled to a current number of seven billion (Bumb and Baanante 1996; UN DESA 2015). Food security was maintained by intensifying agricultural practices in a time period known as the green revolution (Bumb and Baanante 1996; Tilman *et al.* 2001). One of the main farming practises to be improved was fertilisation, in particular N (Olsen 1977; Tilman *et al.* 2001), which in turn led to increased crop yields. Producers, however, readily over-fertilised, which has led to numerous negative environmental and economic effects (Olson 1977; Crews and Peoples 2005). Even though many countries have implemented laws or policies to prevent over-fertilisation of pastures, no such legislation has yet been specified in South Africa (Andrews *et al.* 2007). The recommended N fertilisation rates for grass pastures in South Africa are more than double the legal rate in certain regions of the European Union (Beyers 1973; Andrews *et al.* 2007).

Although legumes have been included into the kikuyu-based pastures of the southern Cape to lower the inorganic N inputs (Swanepoel *et al.* 2015b), producers still apply varying rates of N to grass-legume pastures. Results from trials conducted in New Zealand on perennial ryegrass (*L. perenne*)-white clover (*T. repens)* pastures, indicated that an increase in N fertilisation resulted in a decrease in the white clover component, legume persistence and biologically fixed N (Ledgard and Steele 1992; Harris *et al.* 1996; Ledgard *et al.* 2001). While the effect of N fertilisation on grass-

legume pastures in the southern Cape is unknown, it is hypothesised that excessive N is being applied to the detriment of legume- production, persistence and N fixation. Moreover, N over-fertilisation may also be occurring (Ledgard 2001; Swanepoel *et al.* 2015a). Over-fertilisation threatens the sustainability of cultivated pastures in two ways. Firstly, it poses an environmental threat. If a surplus of N is applied to the soil it can be lost via denitrification, volatilisation or leaching (Ledgard *et al.* 1996; Andrews *et al.* 2007). Nitrogen that is lost can end up in lakes, dams, rivers and seawater (Smil 1999). These nutrients are harmful to the environment and can result in eutrophication (Smil 1999, Tilman *et al.* 2001, Cassman *et al.* 2002). Secondly, the intensive use of N is not economically feasible. The expenditure of N fertilisation of pastures can equate to c. R10 000 to 14 000 ha^{-1} $year^{-1}$. This is a significant expense to farmers and it threatens the economic sustainability of cultivated pastures (Swanepoel *et al.* 2015a, Swanepoel *et al.* 2015b).

The balance of N inputs and losses should be taken into account when determining an appropriate N fertilisation rate. (Ledgard *et al.* 1996; Ledgard 2001). In order to ensure minimal N losses and optimal pasture production, factors including season, rainfall, temperature and pasture growth stage should be accounted for (Ledgard 2001; Di and Cameron 2002). By measuring soil N and comparing it to an optimum soil N threshold value, N fertilisation can be determined (Fessehazion *et al.* 2011). If all factors are accounted for, this fertilisation regime can be referred to as a strategic N application. Legumes, together with strategic N fertilisation, can potentially lower the input of inorganic N fertiliser in cultivated pasture systems (Fessehazion *et al.* 2011; Swanepoel *et al.* 2015b). Although legumes, such as lucerne and clovers, are used as standard forage crops in warm and cool seasons in the southern Cape, respectively, there is a paucity of information on the potential of these crops to reduce N fertilisation under grazing. This is especially true for research on lucerne-kikuyu and kikuyu-perennial ryegrass-clover pastures.

The research questions, therefore, are:

1. How do N fertilisation rates influence soil N dynamics in a grass-legume pasture under grazing?

2. Will N fertilisation rate affect herbage yield of a grass-legume pasture?

3. How will botanical composition of a grass-legume pasture be affected by N fertilisation rate?

4. Will N fertilisation rates affect herbage crude protein content?

1.3 Aims and objectives

Specific objectives during this study will be:

i) to determine the effect of nitrogen fertilisation on total soil nitrogen, total inorganic soil N, potential mineralisable soil N and urease activity,

ii) to determine the effect of nitrogen fertilisation on the herbage yield of grass-legume pastures under no-till management,

iii) to determine if N fertilisation will affect botanical composition,

iv) to determine the effect of N fertilisation on crude protein.

The aim of this study was to determine the effect of N fertilisation regimes on soil as well as pasture parameters for kikuyu-based pastures over-sown with perennial ryegrass-clover and lucerne respectively.

1.4 Structure of the thesis

This thesis consists out of five chapters, prepared according to the guidelines of the *African Journal of Range and Forage Science*.

Following this introductory chapter, where the research problem and aim was outlined, the relevant literature is reviewed in Chapter 2. The emphasis is on the N cycle and the effect of N fertilisation on grass-legume pasture systems. As part of the N cycle, the dynamics between biological N fixation and N fertilisation is highlighted. This chapter aims to holistically evaluate the relationship between N fertilisation and grass-legume pastures and identify research limitations accordingly.

Chapter 3 aimed to determine the effect of different N fertilisation regimes on soil parameters of grass-legume pastures. The soil parameters include: total soil N, total inorganic N, potential mineralisable N and urease activity.

In Chapter 4 the effect of different N fertilisation regimes on herbage parameters of grass-legume pastures will be determined. The herbage parameters include: yield, botanical composition and crude protein.

Chapter 5 concludes research findings, evaluates the limitations and recommends possible future research.

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CHAPTER 2

Literature review

2.1 Nitrogen

2.1.1 Introduction

Nitrogen (N) is one of the most abundant elements on earth, being a large contributor to cellular biomass and making up 78% of the earth's atmospheric gasses (Vance 2001; Ohyama 2010; Stein and Klotz 2016). Nitrogen is one of the 18 essential elements plants require to grow and complete a life cycle (Ohyama 2010). Hydrogen (H), carbon (C), and oxygen (O) are known as the "building blocks" of plant growth (Brady and Weil 2002; Ohyama 2010). These elements are derived from the atmosphere and water (Brady and Weil 2002). The other 15 elements are referred to as plant nutrients and are generally derived from the soil (Brady and Weil 2002). The plant nutrient, N, forms part of almost all plant structures and is a component of chlorophyll, amino acids, protein and hormones (Stitt 1999; Ohyama 2010).

Although N is abundant in the earth's atmosphere, its availability to crop production is limited (Gruber and Galloway 2008; Franche *et al.* 2009). Only leguminous plants can utilise atmospheric N, through the process known as biological N fixation (Ledgard and Steele 1992). Prior to the Haber-Bosch process being developed in 1913, biological N fixation and manure application were the main methods of increasing soil N in crop production systems (Follett *et al.* 2010). The Haber-Bosch process is used to synthesise ammonia (NH_3) from atmospheric N, allowing inorganic N fertilisers to be produced commercially (Follett *et al.* 2010). The use of N fertiliser consequently led to an increase in food production, which in turn could sustain a growing world population (Gruber and Galloway 2008).

2.1.2 Nitrogen cycle

The N cycle (Figure 2.1.1) is a series of biological and chemical reactions that take place when N is cycled between the atmosphere, plants, soil, water and animals (Ledgard *et al.* 1996; Hofman and Van Cleemput 2004). This cycle can be divided into sub-cycles. However, for this specific study, the focus will be on the pedospheric (soil) sub-cycle.

In nature, N occurs in oxidation states ranging from –3 in ammonium (NH_4^+) to +5 in nitrate (NO_3^-) (Hofman and Van Cleemput 2004; Follett *et al.* 2010; Ward *et al.* 2011). The transformation of N from one oxidation state to the next can lead to the loss, gain or redistribution of N in the soil (Figure 2.1.1) (Follett *et al.* 2010).

Nitrogen generally occurs in soil as NO_3^-, NH_4^+ or as part of soil organic matter (SOM) (Hofman and Van Cleemput 2004). Only NO_3^- and NH_4^+ are available for plant uptake, however, most soil N occurs as part of SOM. Mineral-N is incorporated into SOM by a series of microbial conversions, known as immobilisation (Robertson and Groffman 2007), while the mineralisation process releases N from SOM to form NH_4^+. During the process of volatilisation, NH_4^+ is hydrolysed to form NH_3 gas (Meisinger and Jokela 2000). During nitrification, NH_4^+ is reduced to NO_3^- (Ward *et al.* 2011). Nitrate is a mobile anion and can be lost from the soil by leaching. Denitrification further reduces NO_3^- to N_2 gas (Robertson and Groffman 2007). Biological N fixation increases soil NH_4^+ as leguminous plants convert N_2 gas to NH_4^+ (Ledgard and Steele 1992; Hardarson and Atkins 2003).

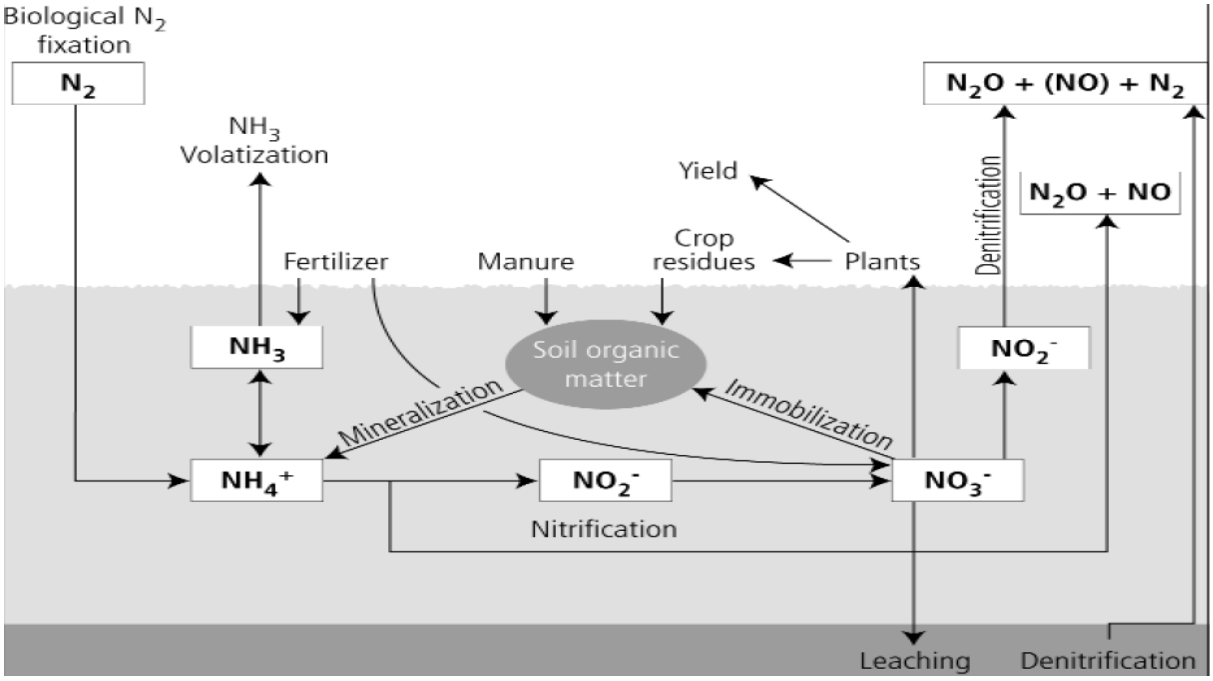

Figure 2.1.1. Simplified version of the N cycle (Adopted from Hofman and Van Cleemput 2004).

2.1.2.1 Nitrogen mineralisation and immobilisation

Soil micro-organisms assimilate SOM as a source of nutrients, C and energy (Hofman and Van Cleemput 2004). These micro-organisms rely on the enzymes urease and amidase to catalyse the release of the nutrient N bound to organic matter (Das and Varma 2011). Urease hydrolyses urea to form NH_3 and carbonic acid (Krajewska 2009). Amidase hydrolyses aliphatic amides to forms NH_3 and carboxylic acid (Frankenberger and Tabatabai 1980). The N released by enzymes is assimilated by micro-organisms and incorporated into cellular components like nucleic acids and proteins (Robertson and Groffman 2007). As micro-organisms assimilate SOM abundant in N, microbial N needs are met and the surplus N is released, or mineralised (Robertson and Groffman 2007). Mineralisation is, therefore, the series of reactions where micro-organisms convert a portion

of the N contained in SOM to the plant available form of NH_4^+ (Hofman and Van Cleemput 2004; Deenik 2006). As micro-organisms consume SOM low in N, additional N has to be utilised from their surroundings. The assimilated N is directly incorporated into microbial biomass, thus immobilised (Robertson and Groffman 2007). Immobilisation is therefore the series of reactions where mineral N is incorporated into an organic N form, which is in turn unavailable to plants (Brady and Weil 2002). The mineralisation and immobilisation processes occur simultaneously in soil. The difference between the total mineralisation and total immobilisation determines which process is dominant. Under net immobilisation inorganic soil N decreases, while under net mineralisation soil N increases (Robertson and Groffman 2007). Furthermore, not all N released from N mineralisation is available for plant uptake. After mineralisation occurs, up to 50% may rapidly be immobilised, leaving only 50% for plant use (Hatch *et al*. 2000).

2.1.2.2 Factors influencing mineralisation and immobilisation

Although the mineralisation and immobilisation processes are primarily controlled by the SOM quantity and quality, factors like soil water and soil temperature can contribute to mineralisation and immobilisation rates (Deenik 2006; Robertson and Groffman 2007).

a) Soil organic matter quantity and quality

The quantity of SOM influences microbial activity rate and therefore determines the mineralisation and immobilisation rate (Robertson and Groffman 2007). Soil organic matter quality controls the equilibrium between gross N immobilisation and gross N mineralisation (Robertson and Groffman 2007). Quality is expressed as the ratio between the availability of C to N. As a general rule, soils with C:N ratios exceeding 25:1 will be prone to immobilisation, and ratios less than 25:1 prone to mineralisation (Honeycutt *et al.* 1988; Hofman and Van Cleemput 2004; Robertson and Groffman 2007). An initial increase in SOM, increases the C:N ratio and results in net N immobilisation. However, over time C:N decreases and net N mineralisation is generally the dominant proses (Kumar and Goh 1999).

Nitrogen fertilisation influences the soil C:N ratio, and therefore affects soil N availability (Kumar and Goh 1999, Zhang *et al.* 2012). Zhang *et al.* 2012 studied the effect of inorganic N fertilisation on N mineralisation under grasslands. Nitrogen was applied at 0, 20, 80 and 160 kg ha^{-1} yr^{-1}. They determined that net N mineralisation increased as N fertilisation increased from 0 to 80 kg N ha^{-1} yr^{-1}. However, there was no significant difference between 80 and 160 kg N ha^{-1} yr^{-1}. Zhang *et al.* (2012) stated that soils receiving more than 80 kg N ha^{-1} yr^{-1} became N saturated, resulting in conditions where N supply surpassed biological demand. Nitrogen saturation of soils, through leaching, can lead to losses of NO_3^-, calcium (Ca) and magnesium (Mg).

Dick *et al.* (1988) determined that an increase in inorganic N fertilisation decreased urease and amidase activity, while Dick (1992) stated that organic N inputs, like manure, usually increases microbial activity and thus increases urease and amidase activity.

b) Temperature and soil moisture

As already mentioned, nitrogen mineralisation and immobilisation rates are related to microbial activity. In turn, microbial activity increases with increased temperatures and is limited near freezing (Jarvis *et al.* 1996, Deenik 2006). Maximum N mineralisation and immobilisation rates occur at soil temperatures between 25 and 37°C (Jarvis *et al.* 1996; Deenik 2006). Under low soil moisture conditions, microbial activity is limited, and therefore N mineralisation and immobilisation rates are low. At soil moisture saturation, only anaerobic microbes are active and thus mineralisation and immobilisation rates are reduced (Jarvis *et al.* 1996; Robertson and Groffman 2007).

Doel *et al.* (1990) researched the influence of soil water and heat units on N mineralisation. Net mineralisation was determined under temperatures of 15, 20 and 25°C and soil water potentials of -0.01, -0.03 and -0.30 MPa. Doel *et al*. (1990) determined that net mineralisation commenced sooner under the 25°C treatment than the 15°C treatment. Additionally, they determined that heat units (degree days) can be used to predict the onset of net mineralisation. The authors also stated that net mineralisation was affected by soil water content. At a constant temperature of 20°C, it was found that the rate of net mineralisation increased as soil water potential increased from -0.03 MPa to -0.01 MPa.

Hatch *et al.* (1991) studied N mineralisation of soil under perennial ryegrass and perennial ryegrass-clover pastures. Nitrogen mineralisation rates in both swards were lower in spring and autumn and higher in summer. In this study the soil temperature at 10 cm depth was positively correlated with average daily N mineralisation rate. Zhang *et al.* (2012) measured net N mineralisation under grasslands and also determined that net N mineralisation was highest in the warmer summer months and lowest in the cooler winter months.

2.1.2.3 Ammonia volatilisation

Ammonia volatilisation occurs when ammonium based constituents are converted to ammonia gas ($NH_{3\,(g)}$) (Equation 1) (Meisinger and Jokela 2000). The equilibrium between NH_4^+ and $NH_{3\,(g)}$ can be predicted by Equation 1.

Equation 1: $NH_4^+ + \text{Heat} \leftrightarrow NH_{3\,(g)} + H^+$

According to the equilibrium equation, the formation of $NH_{3\ (g)}$ is favoured by an increase in temperature, pH and NH_4^+ concentration (Bussink 1992; Brady and Weil 2002; Søgaard *et al.* 2002). Additionally, soil texture and cation exchange capacity (CEC) also influence $NH_{3\ (g)}$ formation.

a) Influence of pH

The relationship between NH_3, NH_4^+ and pH is described by Figure 2.1.2. As soil pH increases above 7, NH_3 increases and NH_4^+ decreases. Ammonia increases by c. 30% when pH rises from 7 to 9. Ammonia is a gas and is unavailable for plant uptake. It is therefore recommended that soils under pasture are maintained between the optimum pH (KCl) of between 5.5 and 6.5.

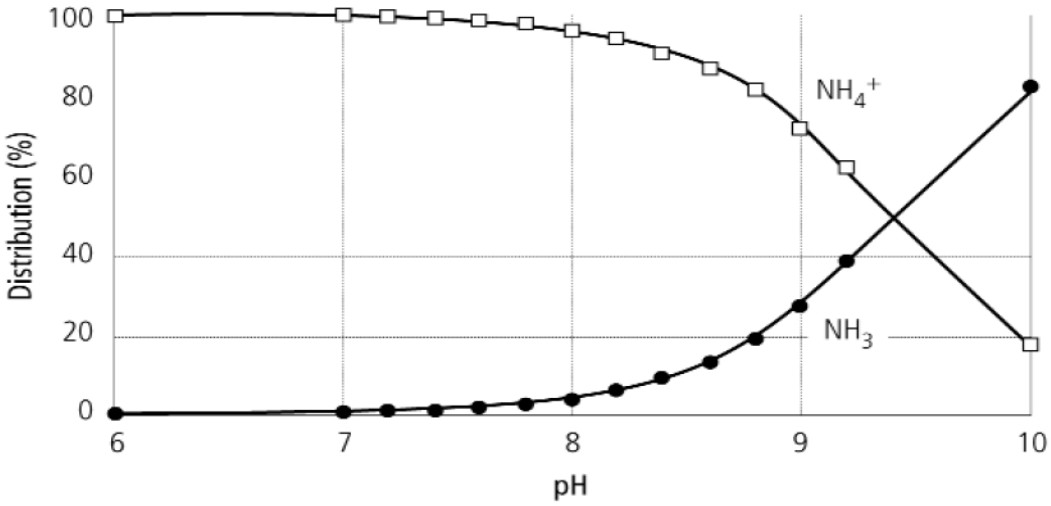

Figure 2.1.2. The relationship between pH and distribution of NH_3 and NH_4^+ (adopted from Court *et al.* 1964)

a) Ammonium concentration

Ammonia losses are increased at higher NH_4^+ concentrations (Equation 1). Ledgard *et al.* (1996) studied soil N losses after N fertiliser (urea) was applied to a perennial ryegrass-clover pasture. They determined that N fertilisation of 0, 225 and 360 kg N ha^{-1} yr^{-1} led to annual NH_3 volatilisation losses of 15, 45 and 63 kg N ha^{-1} yr^{-1} respectively. Bussink (1992) studied NH_3 volatilisation from grazed pastures after calcium ammonium nitrate was applied. Calcium ammonium nitrate was applied at 250 and 550 kg N ha^{-1} yr^{-1}. It was determined that volatilisation increased from 8 to c. 42 when N was applied at 250 kg N ha^{-1} to the 550 kg N ha^{-1} treatment. From these two studies it is clear that the N source, as well as the N application rate, influences ammonia volatilisation. Soils that are subjected to urea will be more prone to volatilisation than when calcium ammonium nitrate is used. Furthermore, an increase in N fertilisation rate will result in an increase in volatilisation.

b) Soil texture and CEC

Obcemea *et al.* (1988) compared NH_3 volatilisation of a clay soil with a CEC of 47 meq 100 g^{-1} soil to a loamy sand soil with a CEC of 5 meq 100 g^{-1} soil. It was determined that up to 30 % more NH_3 was volatilised in the loamy sand than the clay soil. Obcemea *et al.* (1988) concluded that a heavier textured soil with a high CEC adsorbs NH_4^+ ions more efficiently than a light textured soil with a low CEC. Al-Kanani *et al.* (1991) compared a sandy soil to a sandy clay loam soil and confirmed Obcemea *et al.* (1988) findings. Both studies thus determined that an increase in the clay content and CEC of soil reduced N losses to NH_3 volatilisation.

2.1.2.4 Nitrification

Nitrification is the process where NH_4^+ is oxidised to NO_3^- in two phases (Hofman and Van Cleemput 2004). In the first phase the autotrophic bacteria, *nitrosomonas*, oxidizes NH_4^+ to nitrite (NO_2^-). In phase two, NO_2^- is oxidized to NO_3^- by the *nitrobacter* bacteria (Alexander 1977; Hofman and Van Cleemput 2004; Sahrawat 2008). Nitrification is mainly regulated by NH_4^+ availability, soil moisture, temperature and pH (Robertson and Groffman 2007; Sahrawat 2008). Factors controlling the availability of soil NH_4^+ are explained in sections 2.1.2.1 and 2.1.2.3.

The relationship between soil moisture, microbial activity and nitrification is illustrated by Figure 2.1.3 (Linn and Doran 1984). Soil moisture is indicated as water-filled pore space. As water-filled pore space increases, microbial activity and nitrification increases, however, if water-filled pore space exceeds 60%, aeration is limited and microbial activity and nitrification decreases. Sahrawat (2008) states that maximum soil nitrification rate is reached near field water capacity and is at a minimum at saturation. The optimum temperature under which nitrification occurs, ranges between 25°C - 35°C (Brady and Weil 2002; Sahrawat 2008). Nitrification is restricted at a soil pH (KCl) of less than 5. However, as soil pH (KCl) increases above 6, nitrification increases (Sahrawat 1982; Sahrawat *et al.*1985).

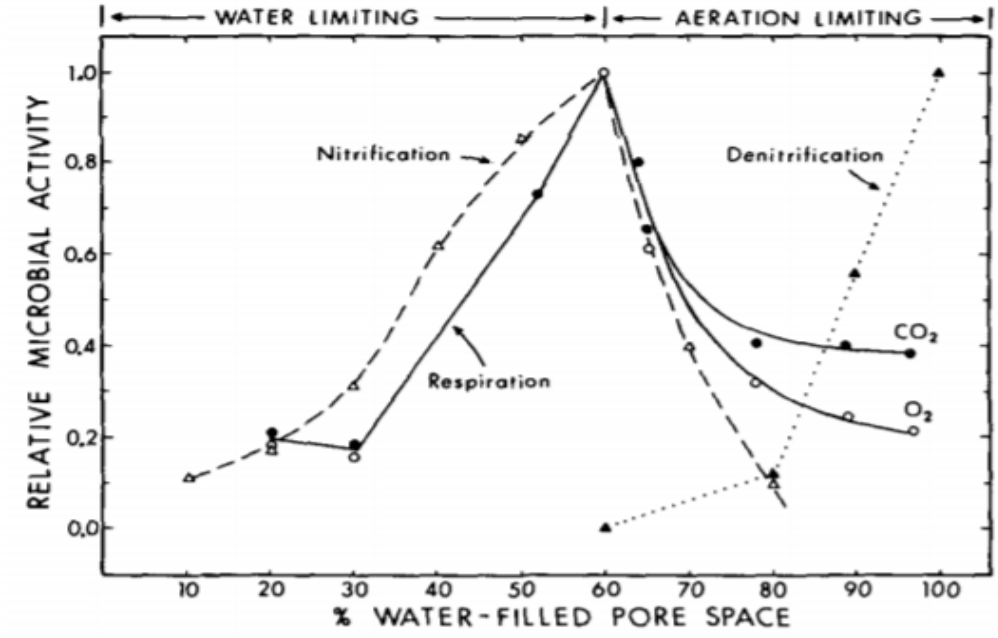

Figure 2.1.3. The relationship between microbial activity and water filled pore space (adopted from Linn and Doran 1984).

2.1.2.5 Denitrification

During denitrification, NO_3^- is reduced to N-gasses (N_2O, NO and N_2) (Zumft 1997; Fowler *et al.* 2013). Although a small amount of soil nitrate is reduced chemically (non-microbial), denitrification primarily occurs due to microbial activity (Brady and Weil 2002). The microbes responsible for denitrification are facultative anaerobic micro-organisms known as denitrifiers (Brady and Weil 2002; Robertson and Groffman 2007). Under aerobic conditions denitrifiers prefer oxygen (O_2) as terminal electron acceptor during respiration. However, when O_2 is limited (anaerobic conditions) denitrifiers utilise NO_3^- (Zumft 1997). Denitrification occurs when water-filled pore space increases above 60%, during which oxygen becomes limited (Figure 2.1.3) (Linn and Doran 1984; De Klein and Van Logtestijn 1996). Denitrification is therefore a prominent process in soils that are waterlogged (Robertson and Groffman 2007).

2.1.2.6 Leaching

Nitrate leaching occurs when negatively charged NO_3^- ions are not retained by positively charged soil colloids, and are subjected to drainage. Nitrate leaching results in NO_3^- being lost from the root zone and transported to ground water, rivers or dams (Di and Cameron 2002; Hofman and Van Cleemput 2004). Drinking water which contains high levels of nitrates can be harmful to human health. The World Health Organisation therefore states that the nitrate concentration of drinking water should not exceed 44 to 50 mg NO_3^- L^{-1} (Di and Cameron 2002; Andrews *et al.* 2007). Under grazed pastures, NO_3^- leaching is generally high due to high N inputs from inorganic N fertiliser and animal excreta (Ledgard *et al.* 1996).

a) Nitrogen fertilisation

Ledgard *et al.* (1996) determined that N fertilisation rates of 0, 225 and 360 kg N ha^{-1} yr^{-1} applied to a perennial ryegrass-white clover pasture (under grazing) led to NO_3^- leaching of 13, 18 and 31 kg N ha^{-1} yr^{-1} respectively. Leaching, therefore, did not differ between 0 and 225 kg N ha^{-1} yr^{-1} application rates. Di and Cameron (2002) reported that when N fertilisation rates, applied to a perennial ryegrass-white clover pasture, exceed 200 kg N ha^{-1} yr^{-1}, leaching generally causes drinking water to exceed the maximum prescribed threshold of 50 mg NO_3^- L^{-1}. Di and Cameron (2002) stated that NO_3^- leaching can be reduced by: i) lowering N inputs, ii) synchronising the N fertilisation rate with pasture N demand, to avoid accumulation of soil NO_3^-, and iii) limiting N fertilisation before or during periods of high leaching risk, for example periods of high rainfall.

b) Animal excreta

Grazing animals return between 60 and 95% of consumed N to the pasture through excreta (Ledgard and Steele 1992, Ledgard 2001). Ledgard *et al.* (1996) reported that as N fertilisation rates of a perennial ryegrass-white clover pasture increased from 0 to 360 kg N ha^{-1} yr^{-1}, N intake by grazing cows increased, and consequently N from excreta increased from 430 to 520 kg N ha^{-1} yr^{-1} respectively. Excreta can be divided into dung and urine (Ledgard 2001). Up to 70% of N that is returned to pastures originates from urine deposits. Urine deposition is localised in small patches that equate to c. 1000 kg N ha^{-1} (Ledgard and Steele 1992; Di and Cameron 2002). Under intensive dairy farming systems, 30% of the grazing area can be subjected to urine deposition (Ledgard and Steele 1992, Di and Cameron 2002). Di and Cameron (2002) reported that N from urine patches generally exceeds the pasture's N demand. The grazing animal component can thus result in localised accumulation of N and increase the potential for leaching on pastures.

2.1.3 Biological N fixation

As result of a symbiotic relationship between leguminous plants and *Rhizobium* bacteria, legumes are able to convert N_2 gas to NH_3 (Hardarson and Atkins 2003; Hofman and Van Cleemput 2004). *Rhizobium* infects the root and induces the formation of nodules (N_2 fixing organs) (Ledgard and Steele 1992). The development of the nodule can be fragmented into three steps. The first step relies on the ability of the bacteria to survive and colonise the soil (Ledgard and Steele 1992). Rhizobia strains in soil are often too low to effectively inoculate legumes and thus roots fail to develop nodules (Hardarson and Atkins 2003). The seeds of forage legumes are thus usually inoculated before planting with a coat of viable rhizobia to ensure effective nodulation (Ledgard and Steele 1992). The second step is the infection of the legume root. Some legume species can only be infected by a few restricted species of bacteria, while others can be infected by more than one (Franche *et al.* 2009). *Rhizobium* induces a deformation of the root hair and by digestion of the cell wall, penetrates the root hair (Sprent 1989; Ledgard and Steele 1992). The final step is the initiation and development of the nodule. The fully developed nodule fixes N gas to form NH_3, which is assimilated to ureides and amides, and translocated to shoots for plant growth (Ledgard and Steele 1992; Hofman and Van Cleemput 2004). The legume in return provides a habitat and a source of energy for the bacteria in form of malate and succinate (Sprent 1989; Hardarson and Atkins 2003).

2.1.3.1 Biological N fixation of a grass-legume pasture

Biological N fixation of a grass-legume pasture is reliant on the dynamic between the grass and legume component. Ledgard and Steele (1992) stated that the biological N fixation of a grass-legume pasture is influenced by the competitive growth of the grass component, the persistence of the legume and the N status of the soil. They stated that biological N fixation is positively correlated with legume production, therefore any factor that favours legume production will lead to an

increase in biological N fixation. Ledgard and Steele (1992) additionally stated that an increase in legume production causes a decrease in grass growth. Stress factors like soil water, temperature, pests and disease will, however, have a larger effect on the legume component. The occurrence of any of these external stress factors will therefore reduce legume production and biological N fixation, and in turn enhance grass dominance.

2.1.3.2 Relationship between soil N and biological N fixation of a grass-legume pasture

Soil N, and the competition between the grass and legume components are associated with a feedback mechanism that regulates biological N fixation (Ledgard 2001). Grass generally grows taller and more vigorously than legumes, therefore, under high soil N levels, grasses have a competitive advantage and dominate the pasture (Ledgard and Steele 1992; Ledgard *et al.* 2001). Under these conditions the feedback mechanism inhibits biological N fixation and the legume substitutes fixed N for inorganic N (Ledgard 2001; Ledgard and Steele 1992). Under low soil N levels, legumes fix more N and have a competitive advantage over the grass (Ledgard 2001; Ledgard *et al.* 2001).

Ledgard *et al.* (2001) studied the outcome of three N fertilisation regimes (0, 200 and 400 kg N ha^{-1} yr^{-1}) on the production and N fixation of a perennial ryegrass-clover pasture. The study found that annual N fixation decreased from 154 kg N ha^{-1} yr^{-1} under the control (0 kg N ha^{-1} yr^{-1}) to 99 and 39 kg N ha^{-1} yr^{-1} under the 200 kg N ha^{-1} yr^{-1} and 400 kg N ha^{-1} yr^{-1} fertilisation regimes respectively. It was estimated that for every kilogram of N applied, on average, annual N fixation decreased by 0.27 kg. This was mostly due to a decline in the contribution of the clover component to the sward. Under high N fertilisation rates, N fixed by clover was increasingly substituted with fertiliser N. In the control treatment, 77% of clover N was derived from biological N fixation, while under the 400 kg N ha^{-1} yr^{-1} treatment only 43% was derived from biological N fixation. Pastures receiving 200 kg N ha^{-1} yr^{-1} and 400 kg N ha^{-1} yr^{-1} did however show a 12 and 25% higher yield compared to the control, respectively. The study undertaken by Ledgard *et al*. (2001) additionally determined that between 61 and 72% of annual fixed N was fixed from spring to summer. As a result, it is concluded that N supply originating from biological N fixation can be sufficient for clover production in warmer months of the year.

The feedback mechanism associated with inorganic N and biological N fixation regulates N inputs from biological N fixation. Consequently, N losses from a grass-legume system are also regulated. Ledgard *et al.* (1996) studied N losses of three N treatments (0, 225 and 360 kg N ha^{-1} yr^{-1}) applied to a perennial ryegrass-white clover pasture. Nitrogen losses via denitrification, leaching and ammonia volatilisation increased with an increase in N input. Ledgard *et al.* (1996) stated that the 225 kg N ha^{-1} yr^{-1} treatment had little effect on biological N fixation and did not result in significant losses in comparison to the 0 kg N ha^{-1} yr^{-1} treatment.

2.1.3.3 Transfer of N from legume to grass

Fixed N is transferred from the legume to the grass via the following pathways: excreta from grazing animals, direct root excretion or legume decomposition (Ledgard and Steele 1992). The amount of N transferred, and pathways used, are dependent on species and environmental factors.

Ledgard (1991) studied N transfer of perennial ryegrass-clover pastures under grazing. He determined that N transfer from cow excreta equated to 60 kg N ha^{-1} yr^{-1}. Excretion from roots and legume decomposition contributed to 70 kg N ha^{-1} yr^{-1}. The transfer of N from excreta was determined by Ledgard (1991) to be 22% of biological N fixation. The transfer of N from animal excretion and legume decomposition collectively equated to 26% of biological N fixation. Høgh-Jensen and Schjoerring (1997) determined that in the first year of production, white clover only transferred 3% of the fixed N to perennial ryegrass. In year two and three, 17 and 22% of fixed N was transferred to the grass component respectively. Louarn *et al.* (2015) compared N transfer of grass- white clover to grass-lucerne pastures. The study was conducted in the absence of grazing animals. They stated that even though lucerne produced 2-fold more yield and fixed more N than white clover, N transferred to the grass was less. White clover transferred c. 50 kg N ha^{-1} yr^{-1} in comparison to c. 20 kg N ha^{-1} yr^{-1} by lucerne. An increase in transferred N was correlated to a decrease in legume population. The decomposition of legume material, especially roots, was therefore related to N release. Louarn *et al.* (2015) stated that white clover has a higher fraction of fine roots and a lower C:N ratio than lucerne. Nitrogen is therefore released more rapidly by white clover than lucerne. Burity *et al.* (1989) estimated N transfer from lucerne to associated grass from a cut trial, under field conditions. The results showed that N transfer increased from 5 kg N ha^{-1} in the first year to c. 20 kg N ha^{-1} in year two and three. In year one N transfer occurred mainly via direct root excretion, whereas in year two and three the decomposition of nodules and roots were the main contributors to N transfer. Peoples and Baldock (2001) reviewed seasonal changes in soil mineral N from subterranean clover (*Trifolium subterraneum*) and medic (*Medicago polymorpha*)-based pastures. Mineral soil N is generally highest in autumn and lowest during spring in these swards. The low mineral N in spring is attributed to high pasture growth rates and thus high N demands during this period. They also state that the increase in soil mineral N in the autumn is due to the decomposition of legume material from the spring of the previous growing season.

Ta and Faris (1988) studied the effects of light intensity and temperature on N transfer of lucerne to timothy grass (*Phleum pratense*) under glasshouse conditions. The authors concluded that day/night temperatures ranging from 16-25/14-20^{o}C were optimal for N fixation and N transfer. It was also found that high light intensities (550 uE m^{-2} sec^{-1}) benefitted N transfer more than low light intensities. In these pastures N transfer was found to be due to lucerne decomposition, as well as direct excretion from roots.

2.2 Kikuyu (*Pennisetum clandestinum*)

2.2.1 General

The natural occurrence of kikuyu is restricted to the plateaux regions of central and east Africa at altitudes ranging between 1950 and 2700 m above sea level (Mears 1970). In these regions the average minimum and maximum temperatures vary between 2 and 22°C. Sporadic frosts occur at night and the annual mean rainfall ranges between 1000 and 1600 mm (Mears 1970).

The C_4 grass species, kikuyu, is a robust perennial which spreads from rhizomes as well as stolons (Marais 2001). Even though kikuyu mainly propagates through vegetative reproduction, propagation through seed may also occur (Mears 1970). Roots of the kikuyu grass are formed at the nodes of rhizomes and stolons (Whyte *et al.* 1968). Leafy braches originate from stolons (Mears 1970). The surfaces of the leaves are hairy. The robust nature of kikuyu allows it to spread aggressively and therefore it can resist stress factors like heavy trampling and grazing (Mears 1970; Botha 2009; García *et al.* 2014).

2.2.2 Growth requirements

2.2.2.1 Water

Generally, C_4 plants have better heat- and drought tolerance than C_3 plants (Crush and Rowarth 2007). Water stress, however, is a growth determining factor for the C_4 grass, kikuyu (Murtagh 1988; Marais 2001). The kikuyu used in South Africa evolved from a high rainfall area and therefore requires c. 850 to 1270 mm rainfall annually (Marais 2001). Murtagh (1988) studied the effect of water supply on kikuyu. He compared evaporation rates of 2 and 5 mm day^{-1} at a soil water potential of -20 kPa and determined that kikuyu growth was 61% lower at 5 mm day^{-1} in comparison to 2 mm day^{-1}. He also stated that kikuyu growth is terminated at a soil water potential of c. -590 and -100 kPa when subjected to evaporation rates of 2 and 5 mm day^{-1} respectively. Well drained, deep soils are therefore preferred for the growth of kikuyu. A reasonable amount of waterlogging is tolerated (Mears 1970; Marias 2001).

2.2.2.2 Temperature

Temperature dictates the growth pattern of kikuyu (Botha 2009). At an air temperature of 25°C kikuyu growth is at a maximum (Murtagh *et al.* 1987). At an air temperature below 10°C, the metabolic process of photosynthesis is supressed and therefore growth is ceased (Marais 2001). For each degree that the average soil temperature drops below 18°C, at a soil depth of 50 mm, kikuyu production will decrease by 11 kg ha^{-1} day^{-1} (Whitney 1974a). Even though the top growth of kikuyu will be killed by frost, stolons will generally be unaffected (Marais 2001). Under South African conditions, the growth of kikuyu increases with increased temperatures from mid-spring

(November) to mid-summer (February). Suboptimal temperatures restrict the production of kikuyu in the winter and spring, and therefore it is dormant in these seasons (Botha *et al.* 2008; Van der Colf *et al.* 2015).

2.2.3 Pasture production

Pure kikuyu swards can yield 29 t DM ha^{-1} yr^{-1} when N is fertilised at 670 kg ha^{-1} yr^{-1} (Henzell 1968). Whitney (1974b) reported that even higher yields (35 t DM ha^{-1} yr^{-1}) could be achieved when N fertilisation rates increases to c. 875 kg N ha^{-1} yr^{-1}. In the southern Cape region of South Africa, Botha *et al.* (2008) determined that a pure kikuyu sward can produce an herbage yield of 13.8 t ha^{-1} yr^{-1} with N fertilisation at 600 kg ha^{-1} yr^{-1}. However, Swanepoel *et al.* (2014b) determined, for the same area, that herbage yield can reach 21 t DM ha^{-1} yr^{-1} at N fertilisation rates of 380 kg ha^{-1} yr^{-1}.

Even though kikuyu can produce high herbage yields, it is poorly distributed throughout the year (Botha *et al.* 2008). Kikuyu produces most of its herbage in the summer and autumn. Growth reaches a minimum in spring and winter and production declines (Marais 2001). Botha *et al.* (2008) determined that a kikuyu sward can produce 33.9 kg DM ha^{-1} day^{-1} in spring, however the growth rate was more than double in the summer and autumn at 67 and 71.6 kg DM ha^{-1} day^{-1}, respectively (Botha *et al.* 2008). Swanepoel *et al.* (2014b) reported that kikuyu production can increase from 1.53 t ha^{-1} in the winter to 7.47 t ha^{-1} in the summer.

2.3 Perennial ryegrass (*Lolium perenne*)

2.3.1 General

Perennial ryegrass originated in the temperate regions of Europe and north Africa (Lamp *et al.* 1990). High nutritional value, palatability, digestibility and tolerance to environmental stresses are beneficial traits of perennial ryegrass (Grogan and Gilliland 2011; Truter *et al.* 2015). It is therefore used as a forage crop in the temperate areas of South Africa, New Zealand, North America and Australia (Lamp *et al.* 2001; Thorogood 2003).

Perennial ryegrass is characterised by shiny hairless leaves that are smooth on the lower surface. The vegetative tillers and nodes form at ground level. The ligules formed by perennial ryegrass are white and translucent (Langer 1979; Lamp *et al*. 2001).

2.3.2 Growth requirements:

2.3.2.1 Temperature

Perennial ryegrass optimally grows at air temperatures ranging between 18 and 20°C (Botha 2009; Chapman *et al.* 2011). Maximum growth rates have been reported between 7 and 20°C, however

average day and night temperatures that exceed 31°C and 25°C, respectively, will limit growth (Thorogood 2003; Chapman *et al.* 2011).

Perennial ryegrass has a high vernalisation requirement. For flowering to occur, temperatures of less than 7°C must be followed by photoperiods longer than 12 hours (Thorogood 2003). In sub-tropical areas, only 5 to 10% of perennial ryegrass tillers become reproductive (Fulkerson *et al.* 1998). Under South African climatic conditions the seeding of perennial ryegrass rarely occurs and persistence beyond the second year after establishment is poor (Donaghy *et al.* 1997).

2.3.2.2 Water

Perennial ryegrass is poorly adapted to conditions of low soil water availability (Thorogood 2003). Even though the minimum annual required rainfall for perennial ryegrass is in the range of c. 460 and 640 mm, it is generally cultivated under irrigation, or where rainfall exceeds 900 mm (Thorogood 2003). Perennial ryegrass tends to have a smaller root density than other temperate grasses and cannot access water deep in the soil profile (Chapman *et al.* 2011). When perennial ryegrass is subjected to a water shortage, tillering, leaf appearance and leaf growth is restricted. Perennial ryegrass has the ability to recover from drought periods to obtain plant-, leaf- and tiller densities similar to those before the water deficient period (Barker *et al.* 1985).

2.3.2.3 Pasture production

Perennial ryegrass monocultures, in the Hontara region of New Zealand, yielded 18.8 t DM ha^{-1} yr^{-1} under an N fertilisation regime of 400 kg N ha^{-1} yr^{-1} (Minneé *et al.* 2010). Wilkins (1991) stated that perennial ryegrass responds increasingly less to N fertilisation rates above 300 kg N yr^{-1}. In South-Africa's southern Cape region, N was applied at 600 kg ha^{-1} yr^{-1} to a pure perennial ryegrass pasture and yields of 14.8 and 14.6 t DM ha^{-1} yr^{-1} were reported in the first and second year of production respectively (Botha and Gerber 2006).

Under South African climatic conditions, the herbage production of perennial ryegrass reaches a maximum in spring and decreases in summer and autumn (Figure 2.3.1). Annual DM production of these pastures generally decreases in successive years after establishment. Under these conditions, perennial ryegrass is re-established either annually or biennially (Donaghy *et al.* 1997). Perennial ryegrass is therefore utilised in combination with other pasture species to achieve optimum distribution of herbage production throughout the year. Van der Colf *et al.* (2015) determined that perennial ryegrass (cv. Bronsyn)-kikuyu (550 kg N ha^{-1} yr^{-1}) pasture can produce a yield of 18 t DM ha^{-1} yr^{-1} (Figure 2.3.1).

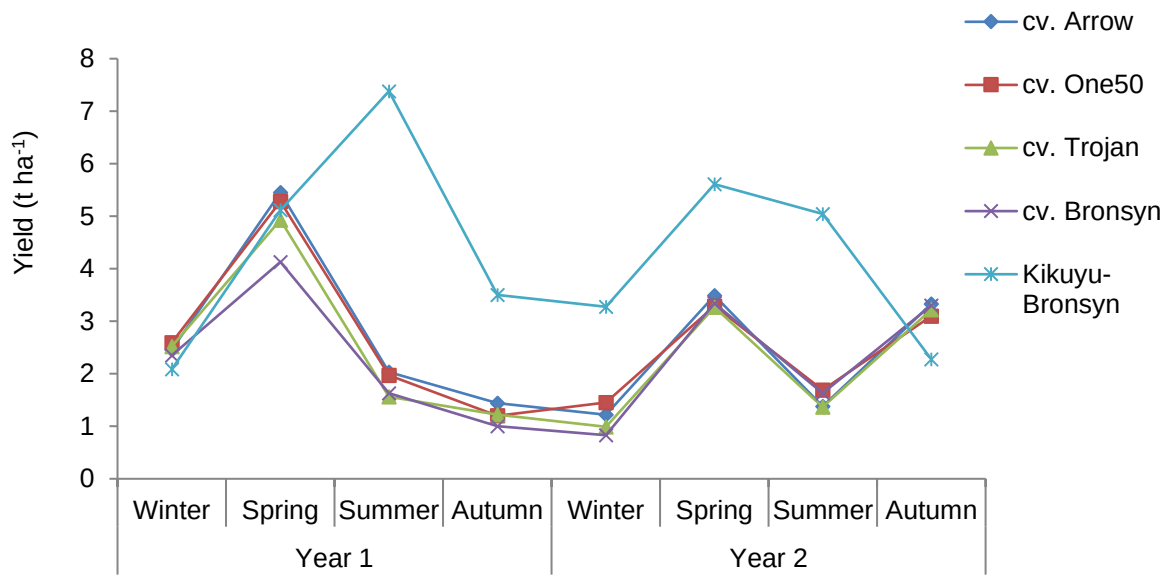

Figure 2.3.1. The seasonal herbage production of perennial ryegrass and kikuyu-perennial ryegrass pastures (adapted from Van der Colf *et al.* 2015 and Van der Colf *et al.* 2016).

2.4 Lucerne (*Medicago sativa*)

2.4.1 General

Lucerne is a perennial, temperate, drought-resistant legume species that is well suited to various climates (Charlton and Stewart 1999; McDonald *et al.* 2003). It originated in central Asia and was first planted in South Africa in the 1850s in the Hexriver valley (Phillips and Miles 1996). Lucerne can yield high quality fodder throughout the year, although the main production period is from spring to early autumn (McDonald *et al.* 2003). Lucerne generally persists for 4 to 6 years and is used for grazing, silage, hay or green fodder (Charlton and Stewart 1999; Brown *et al.* 2003; McDonald *et al.* 2003).

Lucerne is an erect growing perennial legume that grows between 300 and 900 mm high (Charlton and Stewart 1999). Trifoliate leaves, which originate from crown buds, are found on the stems. Roots grow up to 4m deep and even deeper in well drained soils (Langer 1990; Brown *et al.* 2003). Roots are generally concentrated (60 to 70%) to the top 150 mm of the soil profile (Ward *et al.* 2002).

Brown and Moot (2004) studied herbage yield of pure a lucerne sward and reported an average yield (over 5 years) of 21 t DM ha^{-1} yr^{-1}.

2.4.2 Growth requirements

2.4.2.1 Water

Lucerne is more tolerant to drought than white- and red clover (Peterson *et al.* 1992; Brown *et al.* 2003). The taproot system allows lucerne to tolerate drought stress and is therefore often planted under dryland conditions (Brown *et al.* 2005). Holford and Doyle (1978) stated that lucerne can extract water efficiently at soil water potential of -1500 kPa. Under dryland conditions lucerne reduces groundwater recharge by taking up water from deep in the soil profile. Consequently, a rise in water table and salinisation is avoided (Crawford and Macfarlane 1995; Ward *et al.* 2002). Waterlogged soils can however weaken or kill lucerne. Lucerne can survive waterlogged soils for up to 15 days, however, when waterlogging is accompanied by high temperatures this period is reduced (Heinrichs 1970; McDonald 2003).

Brown *et al.* (2003) determined that lucerne had a water use efficiency of 46 kg DM ha^{-1} mm^{-1}, which is similar to chicory and red clover. Tonmukayakul (2009) compared water use efficiency of lucerne to cocksfoot-clover and ryegrass-clover pastures. He determined that lucerne had a higher water use efficiency (21 kg DM ha^{-1} mm^{-1} yr^{-1}) than the various grass-clover mixtures (9 to 15 kg DM ha^{-1} mm^{-1} yr^{-1}). Sheaffer *et al.* (1988) stated that for every ton (DM) of lucerne that is produced, evaporation is between 50 to 90 mm ha^{-1}.

2.4.2.2 Temperature

Lucerne germinates best at temperatures ranging between 20 and 25°C (McDonald *et al.* 2003). Teuber *et al.* (1987) stated that the ideal temperature for lucerne seedlings to develop is between 20 and 22°C. Kendall *et al.* (1994) studied the effect of temperature on the growth of lucerne between the age of 2 and 4 weeks. They determined that the optimum temperature for shoot and root growth was 27°C and 25°C respectively. Temperatures below 10 or above 40 will negatively influence lucerne yield (McDonald *et al.* 2003).

2.5 White clover (*Trifolium repens L.*)

2.5.1 General

For the temperate regions of the world, white clover serves as an important pasture legume (Frame and Newbould 1986). Although white clover is generally described as a perennial, in some management and climatic conditions it acts as an annual (Hollowell 1966; Turkington and Burdon 1983). It is characterised by a high nutritional value and N fixing ability. It is therefore commonly incorporated with temperate grasses as mixed swards (Frame and Newbould 1986).

The primary seedling develops a shallow taproot system, thereafter adventitious roots are formed at the nodes of stolons (Frame and Newbould 1986). Each stolon develops into an individual plant once the primary taproot dies off and the plant is supported by the adventitious roots. The persistence, replacement and development of the stolons are necessary for the survival and spreading of white clover (Frame and Newbould 1986).

2.5.2 Growth requirements

2.5.2.1 Water

Even though the adventitious root system of white clover makes it more susceptible to drought stress than lucerne and red clover, it is has a better tolerance of waterlogging. Heinrichs (1970) determined that white clover can tolerate waterlogged conditions for 20 days, which is 5 days longer than lucerne and red clover.

Hofmann *et al.* (2007) studied white clover's response to drought conditions. White clover plants were subjected to a soil water content of either 5% below field capacity (well-watered treatment) or 5% above permanent wilting point (drought treatment) for four weeks. They determined that DM production as well as photosynthesis was c. 50% lower in plants subjected to the drought treatment than well-watered plants. Ballizany *et al.* (2012) also studied white clover response to drought. The well-watered treatment was maintained at field capacity (38% volumetric soil water content) and the drought treatment was kept just above wilting point (10% volumetric soil water content) for nine weeks. It was determined that the plants subjected to drought conditions produced 21% less DM than the well-watered plants.

2.5.2.2 Temperature

In white clover, growth and N fixation take place between 9 to 27^{o}C. The optimum temperature for these processes is 25^{o}C (Frame and Newbould 1986; Chapman *et al.* 2011).

The low root to shoot ratio of white clover makes it vulnerable to high temperatures and dry conditions (Brock and Hay 2001). Stolon death can be caused by high temperatures in summer months (Hay and Chapman 1984). The persistence of white clover is therefore determined by summer temperatures (Frame and Newbould 1986; Brock and Hay 2001). Archer and Robinson (1989) examined the relationship between soil moisture, temperature and survival probability of white clover grown on a podzolic soil in New South Wales, Australia. They found that a soil water content lower than 40% of field water capacity, accompanied by an average weekly maximum temperature of more than 20^{o}C, reduced white clover survival rate significantly.

2.6 Red clover (*Trifolium pratense*)

2.6.1 General

Red clover is native to south-eastern Europe (Taylor and Quesenberry 1996). Under temperate grazing regimes red clover is included with temperate grasses and white clover to ensure high quality fodder in the summer (Hay and Ryan 1989).

Red clover is a tap-rooted leguminous plant. In well drained soils, roots grow up to 3 m deep. The stems originate from the crown and can grow 800 mm high. Foliage is described as light green and hairy. Flowers can consist out of 300 florets that are usually pollinated through long tongued insects like bumblebees (Bowley *et al.* 1984; Taylor and Quesenberry 1996). The yield of red clover is maintained by the number of stems per plant, which generally increases over time, however, plant densities generally decrease due to disease or negative environmental conditions. Consequently red clover has to be re-established every two years in order to maintain plant densities and optimal yields (Bowley *et al.* 1984).

2.6.2 Growth requirements

2.6.2.1 Water

Red clover, like lucerne, utilises soil water efficiently due to a deep taproot system. Red clover's soil water use varies between 400 and 600 kg water kg^{-1} DM (Bowley *et al.* 1984). Brown *et al.* (2003) stated that the water use efficiency of red clover was c. 46 kg DM ha^{-1} mm^{-1}. It is estimated that red clover can reach a maximum water uptake of 5 mm day^{-1} on a sandy loam soil (Bowley *et al.* 1984).

Although red clover starts to indicate water stress at a soil water potential of -0.1 MPa, wilting will occur at a soil water potential between -0.8 and -1.2 MPa (Hardie and Leyton 1981). Red clover can survive waterlogging for up 15 days, however, herbage yields could decrease by more than 50% if the soil is waterlogged for more than 8 days (Heinrichs 1970; Bowley *et al.* 1984).

2.6.2.2 Temperature

Although red clover can survive temperatures ranging between 7 and 38°C, it grows optimally between 20 and 27°C (Fergus and Hollowell 1960; Bowley *et al.* 1984). Seedlings will die if temperatures reach 40°C (Fergus and Hollowell 1960). When temperatures are raised above optimal levels, roots are affected more negatively than shoots (Bowley *et al.* 1984). A raised temperature leads to an increase in respiration and a decrease in total carbohydrates. Subsequently, red clover becomes more susceptible to diseases and therefore winter persistence will decrease (Bowley *et al.* 1984).

2.7 Grass-legume pastures

2.7.1 Interaction with nitrogen

Nitrogen fertilisation can dictate the growth of legumes in a grass-legume pasture. Not only can inorganic N inputs decrease legume competition, it also affects legume morphology and persistence (Ledgard and Steele 1992).

Harris *et al.* (1996) studied the effect of N on ryegrass-clover pastures. They determined that N application rates of 0, 200 and 400 kg N ha^{-1} yr^{-1} resulted in a clover component of 15.4, 14.9 and 6.8% respectively. Clover density measurements coincided with clover component. Plant densities decreased from 438 plants m^{-2} under 0 kg N ha^{-1} yr^{-1} to 227 and 26 plants m^{-2} under 200 and 400 kg N ha^{-1} yr^{-1} respectively. Harris *et al.* (1996) also stated that the higher N rates (400 kg N ha^{-1} yr^{-1}) resulted in plants with fewer stolons and auxiliary buds.

Murphy *et al.* (1986) studied the effect of a once-off N application (50 kg N ha^{-1} yr^{-1}) on the early-season forage production of a perennial ryegrass-white clover pasture. They found that the average annual clover component was 15.2% when 50 kg N ha^{-1} yr^{-1} was applied and 21.3% for the control (0 kg N ha^{-1} yr^{-1}). This coincided with the findings of Shiel *et al.* (1999) that stated that the clover component of a ryegrass-clover pasture decreased with increased N fertilisation rates. Clark and Harris (1996) developed a simulation model which can be used to predict the effect of N fertilisation rate on clover content, yield and forage quality. According to the model, for a ryegrass-clover pasture to produce optimal yield and forage quality, a clover component of 30 to 40% must be accompanied by a N fertiliser regime of 100 to 200 kg N ha^{-1} yr^{-1}.

2.7.2 Pasture production

Van der Colf (2016) and Fourie (2015) evaluated the DM production of kikuyu-perennial ryegrass-clover and kikuyu-lucerne pastures respectively. The kikuyu-perennial ryegrass-clover pasture received a once-off N application of 50 kg N ha^{-1} in winter and yielded 16 t DM ha^{-1} yr^{-1}. The kikuyu-lucerne pasture received no N and yielded c. 19 t DM ha^{-1} yr^{-1}. For both the kikuyu-lucerne and kikuyu-perennial ryegrass-clover pasture types, the herbage yield was highest in summer during the first year and lowest during the winter (Figure 2.7.1). Botha *et al*. (2008) also found that a kikuyu-perennial ryegrass-clover pasture yielded 15.9 t DM ha^{-1} yr^{-1}, after no N was applied.

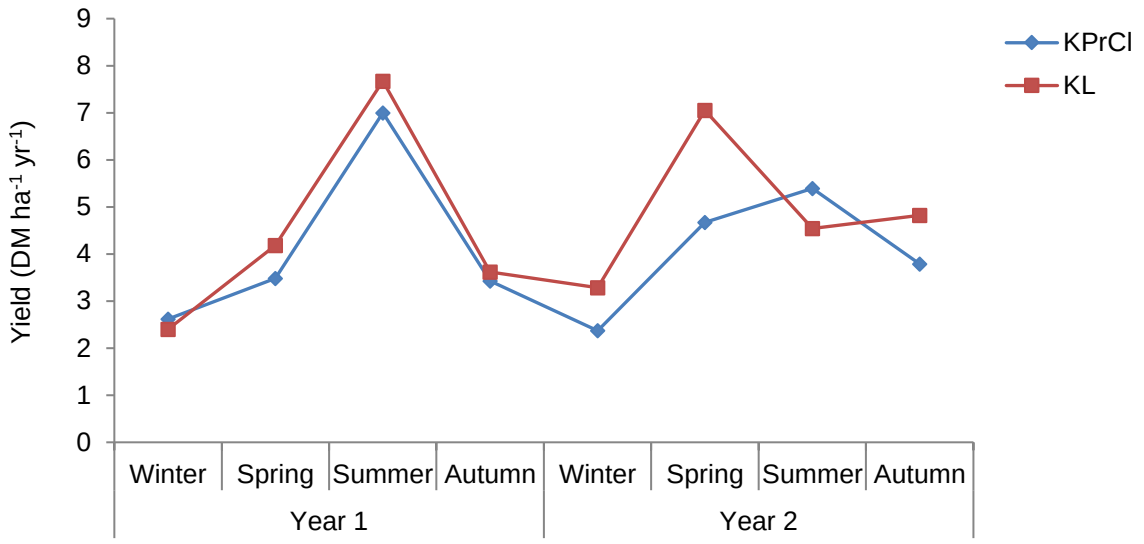

Figure 2.7.1. The seasonal DM production of kikuyu-perennial ryegrass-clover (KPrCl) and kikuyu-lucerne (KL) pastures (Adapted from Fourie 2015; Van der Colf 2016).

Botha and Gerber (2006) measured herbage production of perennial ryegrass-clover and clover pastures over-sown with perennial ryegrass (cv. Bronsyn). Perennial ryegrass was over-sown in the autumn. Both pasture types received no N during the trial period. They determined that the clover pasture had a lower yield than the perennial ryegrass-clover pasture in all seasons except summer.

Shiel *et al.* (1999) reported that perennial ryegrass-clover pastures produced maximum yields when N was fertilised at 300 kg N ha^{-1} yr^{-1}. Ledgard *et al.* (2001) stated that a perennial ryegrass-clover pasture that received 200 and 400 kg N ha^{-1} yr^{-1} can out yield the same pasture that receives no N by 12 and 25%, respectively. They determined that pastures that received 0, 200 and 400 kg N ha^{-1} yr^{-1} yielded 16.4, 18.5 and 20.6 t DM ha^{-1} yr^{-1} respectively. They additionally determined that for every kilogram of N applied, pasture yield increased by 10 kg of DM.

2.7.3 Nitrogen uptake

The inefficient use of N fertiliser can hinder the economic and environmental sustainability of pasture systems (Abassi *et al.* 2005). For N to be utilised efficiently, factors including species type, soil type, climate and season must be taken into account before N is fertilised (Abassi *et al.* 2005).

Botha *et al.* (2008) determined seasonal crude protein content of kikuyu-based pastures. Four pasture types where established namely: kikuyu, kikuyu-annual ryegrass, kikuyu-clover and kikuyu-perennial ryegrass-clover. Grass-only pastures received 600 kg N ha^{-1} yr^{-1} while pasture that had a clover component received no N. Botha *et al.* (2008) stated that the clover-dominant pastures continuously had higher crude protein contents than grass-only pastures. They also determined that the kikuyu-perennial ryegrass-clover pasture had higher crude protein content in

the spring and winter (22.8 to 23.8%) than in the summer and autumn (18.5 to 18.4%). Ledgard *et al.* (2001) measured clover N concentration. The clover was established as a perennial ryegrass-clover pasture and subjected to N fertilisation treatments of 0, 200 and 400 kg N ha^{-1} yr^{-1}. They determined that herbage N concentration was not affected by N fertilisation treatments in winter, spring, summer and autumn.

Fourie (2015) measured the seasonal crude protein content of kikuyu-lucerne pastures. The crude protein of the lucerne was highest in the winter (32%) and lowest in the summer (23%). Kikuyu's crude protein content varied between 14 and 18% in spring and summer, and from winter to autumn crude protein varied between 20 and 23%.

Pasture nitrate concentration usually increases as N fertilisation increases (Smith and Sund 1965; Shiel *et al.* 1999). Generally, nitrate is accumulated in forage due to nitrate supply that surpasses biologic demand. Shiel *et al.* (1999) studied the effect of N fertilisation rates on the forage nitrate concentration of perennial ryegrass-white clover and pure perennial ryegrass pastures. They determined that the perennial ryegrass-white clover pasture continuously had higher nitrate content than the perennial ryegrass pasture at the same N fertilisation rate.

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CHAPTER 3

Variable nitrogen fertilisation and soil nitrogen dynamics of kikuyu-based pastures over-sown with perennial ryegrass and legumes

3.1 Introduction

Soil nitrogen (N) is balanced between N inputs and losses (Ledgard 2001; Brady and Weil 2002). For pastoral soils under grazing, N is lost through removal of herbage through grazing, denitrification, leaching and volatilisation (Ledgard 2001). In commercial agriculture, N lost under grass pastures, is mainly replenished by N fertiliser inputs (Ball and Ryden 1984; Ledgard and Steele 1992). Kikuyu (*Pennisetum clandestinum*)-ryegrass (*Loliuim spp.*) pastures of the southern Cape region of South Africa require up to 600 kg N ha^{-1} yr^{-1} to produce a yield of 18 t dry matter (DM) ha^{-1} yr^{-1} (Botha *et al.* 2008). To reduce high inorganic N inputs, legumes are incorporated into these pastures. Legumes fix atmospheric N and contribute to the soil N balance via biological N fixation (BNF) (Ledgard and Steele 1992; Hardarson and Atkins 2003). The relationship between inorganic N and BNF influences soil N levels (Ledgard *et al*. 1996; Ledgard 2001; Ledgard *et al.* 2001). Biological N fixation in a grass-legume pasture system is regulated by a feedback mechanism associated with inorganic soil N content and competition between the legume and grass component (Ledgard and steele 1992; Ledgard 2001). Under low soil N levels, legumes derive most N from BNF and therefore dominate the pasture. Under high inorganic N levels, grasses outcompete the legumes and inhibit BNF. Consequently, legumes substitute fixed N for inorganic N (Ledgard and Steele 1992; Ledgard 2001). Ledgard *et al.* (2001) determined that a ryegrass-clover pasture that received 0 kg N ha^{-1} yr^{-1} fixed 154 kg N ha^{-1} yr^{-1}, whereas the same pasture that received 200 and 400 kg N ha^{-1} yr^{-1} fixed 99 and 39 kg N ha^{-1} yr^{-1}, respectively. The BNF predominantly took place from summer to spring and up to 70% of the N can be fixed by a ryegrass-clover (*Trifolium spp.*) pasture in this period.

Due to the feedback mechanism between inorganic N and BNF, any factor that influences inorganic soil N will influence BNF. This feedback mechanism therefore also regulates N losses from a grass-legume system (Ledgard *et al.* 1996; Ledgard 2001). Ledgard *et al.* (1996) determined that denitrification, ammonia volatilisation and leaching from a ryegrass-clover pasture increased as N fertilisation increased. Leaching increased three-fold as inorganic N input increased from 0 to 360 kg N ha^{-1} yr^{-1}.

Although grass-legume pastures play an integral role in the dairy producing area of the southern Cape, N fertilisation guidelines for grass-legume pasture mixes in this region do not explicitly exist. To minimise N losses and optimise pasture production as well as BNF, strategic N fertilisation

should be considered. The aim of this study was to determine the effect of different N fertilisation regimes on the total soil N, total inorganic soil N, potentially mineralisable N (PMN) and urease activity of kikuyu-lucerne (*Medicago sativa*) and kikuyu-perennial ryegrass-clover pastures in the southern Cape.

3.2 Materials and methods

3.2.1 Experimental site

Research was conducted on the Outeniqua Research Farm of the Western Cape Department of Agriculture, situated near George (Western Cape, South Africa). The region has a temperate climate, receiving its rainfall throughout the year in increments of 40 to 70 mm per month and a long-term annual rainfall of 728 mm. July is the coolest month, with an average daily minimum and maximum temperature fluctuating between 7 and 18°C, respectively. February is the warmest month and minimum and maximum temperatures vary between 15 and 25°C, respectively (ARC-ISCW 2014).

The study was conducted on existing minimum-tillage kikuyu-based pastures that were divided into two separate sites. A pasture type was allocated to each site. Pasture types were: kikuyu over-sown with lucerne (cv. WL 375) (KL) and kikuyu over-sown with perennial ryegrass (cv. Arrow) and clover (KPrCl). The clover was over-sown as 50% red clover (*T. pratense* cv. Borduro) and 50% white clover (*T. repens* cv. Storm). Each site comprised 0.9 ha. Although the soil on both sites was similar the different pasture types were established on separate sites due to significant differences in irrigation and grazing management (see section 3.2.4; 3.2.5).

Research was carried out on a podzolic soil (IUSS working group 2006). According to the Soil Classification Working Group (1991) the soil is classified as a Witfontein 1100 (Table 3.2.1).

Table 3.2.1. The description of soil- horizons and texture at different depths, found at experimental site**.**

Horizons	**Depth (mm)**	**Sand (%)**	**Slit (%)**	**Clay (%)**
Orthic A	0-350	84	8	8
Podzol B	350-500	76	14	10
Unconsolidated material with signs of wetness	500-800	78	12	10

3.2.2 Experimental design and treatments

On each site, i.e. two pasture types, a randomised block design was used with five N fertilisation treatments replicated in four blocks. Thus, each site contained 20 experimental units. Plots had dimensions of 15 m x 30 m and each site amounted to 0.9 ha. The N treatments consisted of four

fixed application rates namely: 0, 20, 40 and 60 kg N ha^{-1} and one variable rate referred to as a variable N application (N_{var}). Nitrogen fertilisation treatments were applied after grazing. Nitrogen fertiliser was applied by hand in the form of limestone ammonium-nitrate (LAN). The KL and KPrCl pasture types were grazed eight and nine times per year respectively.

Wetting front detectors (WFDs) were used to collect soil water on the N_{var} plots. The soil solution N could be measured from the soil water and subsequently a variable N application rate could be determined (Fessehazion *et al.* 2011). A WFD is a funnel shaped passive lysimeter that is planted in the soil to incept soil water percolating at a certain depth (Stirzaker and Hutchinson 2005). As irrigation or rainwater infiltrates the soil it is intercepted by the wide end of the WFD funnel. If water proceeds to infiltrate, the soil at the narrow end of the funnel becomes saturated. The WFD is designed in such a manner that the soil at the base of the WFD is saturated at the same depth as the soil outside the WFD. At saturation (-2 to -3 kPa) free water is caught in a reservoir. Water in the reservoir activates floats that launch an aboveground flag. Water in the reservoir can then be extracted using a syringe (Stirzaker 2003).

Two WFDs were planted at each N_{var} plot at a depth of 150 mm and 300 mm. When the WFDs' flags launched after a rainfall or irrigation event, the water was extracted and nitrate concentration was determined. Nitrate concentration was measured by a LAQUAtwin water quality meter and by nitrate strips. The average soil solution nitrate concentration measured from the 150 mm WFD during the previous growing cycle was used to determine the N_{var} application rate. Variable N application guidelines were based on the work of Fessehazion *et al.* (2011) from June 2016 to October 2016. However, as these specified N rates resulted in no N fertiliser application, and poor pasture production, the guidelines were adapted from October 2016 after which the following N rates were used: when nitrate concentration measured less than 50 mg L^{-1}, 50 kg ha^{-1} N was applied to the plot. If 50 to 75 mg L^{-1} was measured, 25 kg ha^{-1} N was applied and if more than 75 mg L^{-1} was measured no N was applied. For the KPrCl pasture type, however, only one N_{var} plot received N throughout the whole trial period. This application was at 25 kg N ha^{-1} and was applied in October. For the KL pasture type, N was fertilised at three N_{var} plots during the month of October. These plots were fertilised with 25, 25 and 50 kg N ha^{-1}, respectively.

3.2.3 Seed treatment and establishment

Legume seed was treated with an insecticide and fungicide fourteen days before planting (Table 3.2.2). Seeds were treated with an insecticide for protection against black sand mite (*Halotydeus destructor*) and lucerne flea (*Sminthuris viridis*) (Smit 1964; Botha 2003). The fungicide was used for protection against *Pythium, Phytophthora* and *Fusarium* spp. (Langenhoven 1986).

Table 3.2.2. The pesticide type, active ingredient, commercial product and dosage rate used to treat lucerne and clover seeds before planting.

Pesticide	Active ingredient	Commercial product	Dosage rate
Insecticide	Dimethoate (organophosphate)	Dimethoate	600 mL[1] 100 kg^{-1} seed
Fungicide	Mefenoxam (phenylamide)	Apron XL	50 mL 100 kg^{-1} seed

[1]The 600 mL of Dimethoate was diluted with 4 L of water.

On the day of planting, the lucerne and clover seeds were inoculated with host-specific *Rhizobium* to ensure nodulation (Ledgard and Steele 1992). The KPrCl pasture type was over-sown in year one and two (11 April 2016 and 30 March 2017). The KL pasture type was only over-sown in year one (11 April 2016). Prior to establishment both pasture sites were grazed to 50 mm above ground level and subsequently mulched to ground level with a 1.6 m Nobili mulcher (Botha *et al.* 2008). The KL pasture type was established by over-sowing lucerne into the mulched kikuyu sward at 15 kg ha^{-1} using an Aitchison minimum-tillage seed drill. The KPrCl pasture type was established by over-sowing perennial ryegrass, white clover and red clover into the kikuyu sward as a mixture at 12, 4 and 4 kg ha^{-1}, respectively (Botha *et al.* 2008; Fourie 2015). After each pasture type was planted the site was rolled with a Cambridge-type roller.

3.2.4 Irrigation scheduling

Permanent overhead sprinklers were used to irrigate the trial site. The sprinkler heads were spaced 15 m apart and aligned in a staggered conformation. Tensiometers were used to schedule irrigation. The tensiometers were planted at 200 mm and 150 mm for the KL and KPrCl sites, respectively. Irrigation was scheduled to maintain a soil matrix potential between -25 and -10 kPa (Botha 2002).

3.2.5 Grazing and pasture allocation

A disc pasture meter was used to estimate herbage on offer to cows by measuring herbage height and density before grazing. Subsequently, the herbage on offer (kg DM ha^{-1}) per plot was estimated and Jersey cows were allocated to the pasture accordingly (Stockdale 1984; Van der Colf 2011). Pastures were strip grazed to a height of 50 mm. The recommended grazing interval for the KPrCl and KL pasture types are 28 and 35 days respectively (Botha 2003; Botha *et al.* 2008; Fourie 2015). However, due to lower growth rates in the cooler seasons (winter and autumn), grazing varied between 27 and 35 days for the KPrCl pasture and between 32 and 42 days for the KL pasture type. Disc pasture meter readings were also taken after grazing to estimate the herbage removal by cows.

3.2.6 Soil sampling and analyses

Soil cores were taken monthly, prior to grazing, to a depth of 100 mm and every third month to 300 mm deep. At least five subsamples were taken per plot, using a soil auger (core length 145 mm and diameter 70 mm), and mixed thoroughly to obtain a representative sample for each depth. Soil samples that were obtained to determine ammonia-N, nitrate-N and soil C were stored at room temperature and out of direct sunlight until analysed. The soil samples used to determine total N and PMN were kept cool (5^{o}C) until they could be weighed, after which the samples were dried (40 ^{o}C for 48 hours) and weighed again. The wet and dry weights were used to determine gravimetric soil water content. The average soil water content for the KPrCl and KL pasture type for each sampling event is reported in Figure 3.2.1 and Figure 3.2.2, respectively. After weighing the dry samples, the soil samples were passed thought a 2 mm sieve and kept at 5^{o}C until analysed.

Organic C was determined by dichromateous digestion with the Walkley-Black procedure (Walkley 1935). Total soil N was determined by the total combustion methods with use of a Leco Truspec® CN N analyser (Wright and Bailey 2001). The idophenol-blue (Keeney *et al.* 1982) and salicyclic acid (Cataldo *et al.* 1975) methods were used to determine ammonium and nitrate content, respectively. Subsequently nitrate and ammonium contents were summed to calculate total soil mineral N content. Aerobic incubation was used to determine N mineralisation at 75% field water capacity and 20°C for 7 days. Urease activity was determined by incubating the soil with urea according to the procedure of Kandeler and Gerber (1988).

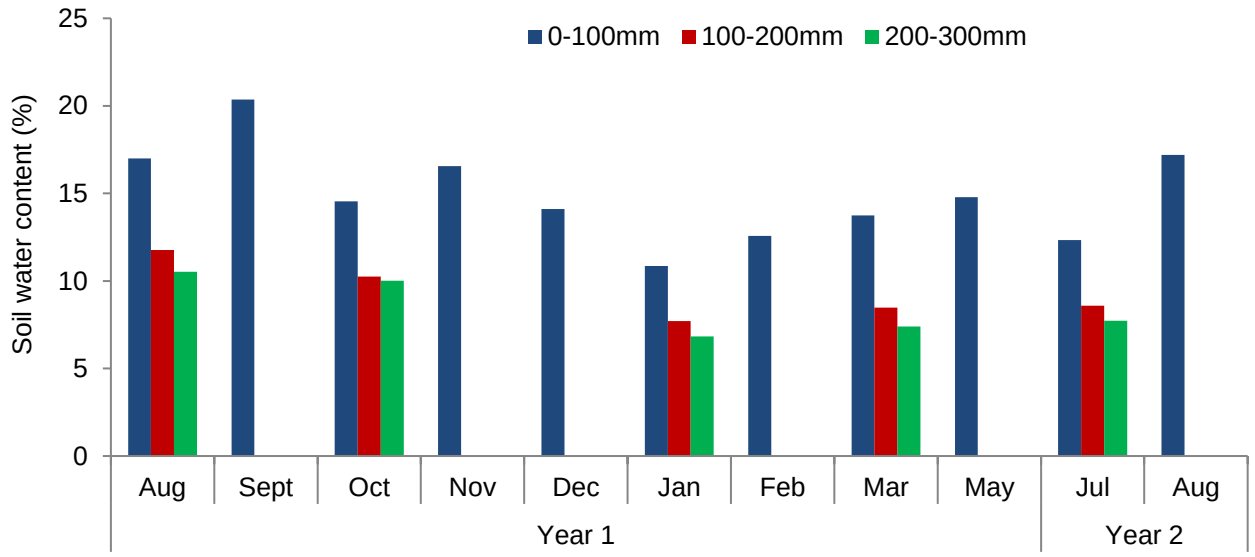

Figure 3.2.1. The average soil water content (gravimetric) for the kikuyu-perennial ryegrass-clover pasture site on the day of sampling. The soil water content is reported for depths of 0-100, 100-200 and 200-300 mm.

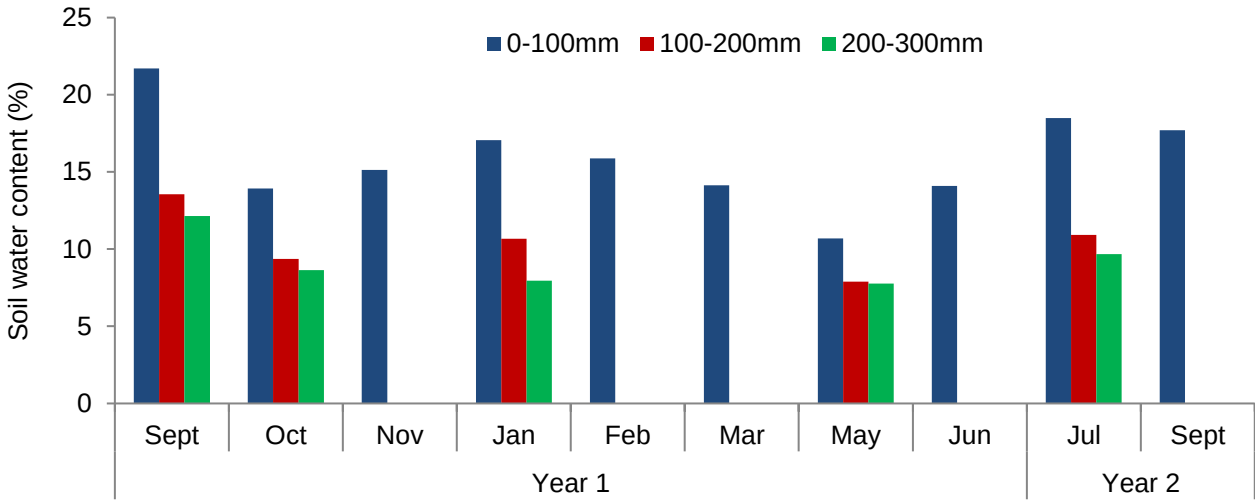

Figure 3.2.2. The average soil water content (gravimetric) for the kikuyu-lucerne pasture site on the day of sampling. The soil water content is reported for depths of 0-100, 100-200 and 200-300 mm.

3.2.7 Statistical analysis

The Restricted Maximum Likelihood (REML) procedure was followed using Variance Estimation, Precision and Comparison (VEPAC) package of STATISTICA (Dell Inc. 2016). The fixed effects were treatment, date and the interaction between treatment and date. Compensation for random effects included effects of block, the cross between block and treatment and the cross between block and date. Normality of residuals were tested for constant treatment variables. The treatment means were compared by carrying out a student t-test at a 5% significance level.

3.3 Results and discussions

3.3.1 Kikuyu-perennial ryegrass-clover soil nitrate

The WFD is an apparatus that intercepts soil water when soil water potential is between -2 to -3 kPa. Because irrigation was managed to sustain soil water potential between -25 and -10 kPa, potential rarely increased above -3 kPa. As result water could only be extracted from the WFDs after certain rainfall events. Consequently, not enough data was collected to perform statistical analyses. The data can still be interpreted and add value to this study. Figure 3.3.1 illustrates the average nitrate concentration measured from water extracted from the 150 and 300 mm WFDs.

For a KPrCl pasture, roots are mostly concentrated in the top 150 mm of soil (Collins *et al*. 2003). The nitrate concentration measured by the 150 mm WFD can therefore be used to indicate plant available nitrate. The nitrate measured form the 300 mm WFD can in turn be interpreted as the nitrate leached out the root zone and largely unavailable for plant use. The nitrate concentration measured from the 150 mm WFD varied between 60 (August year 1) and 177 mg L^{-1} (March year 1). The nitrate measured from the 300 mm WFD varied between 60 (October year 1) and 155 mg

L^{-1} (March year 1). The N application in October might be the reason for the increase in nitrate concentration from October to November in both the 150 and 300mm WFD.

According to the World Health Organisation if the nitrate concentration in drinking water exceeds 50 mg NO_3^- L^{-1} it is deemed unsafe for human consumption (Di and Cameron 2002; Andrews *et al.* 2007). The nitrate concentration measured form the 300 mm WFD consistently exceeded 50 mg L^{-1}. Since nitrate deeper than 300 mm is mostly unavailable for plant use, it may leach out of the soil profile and end up in ground water. The nitrate, measured at this depth, throughout the study may potentially lead to ground water nitrate pollution and pose an environmental threat.

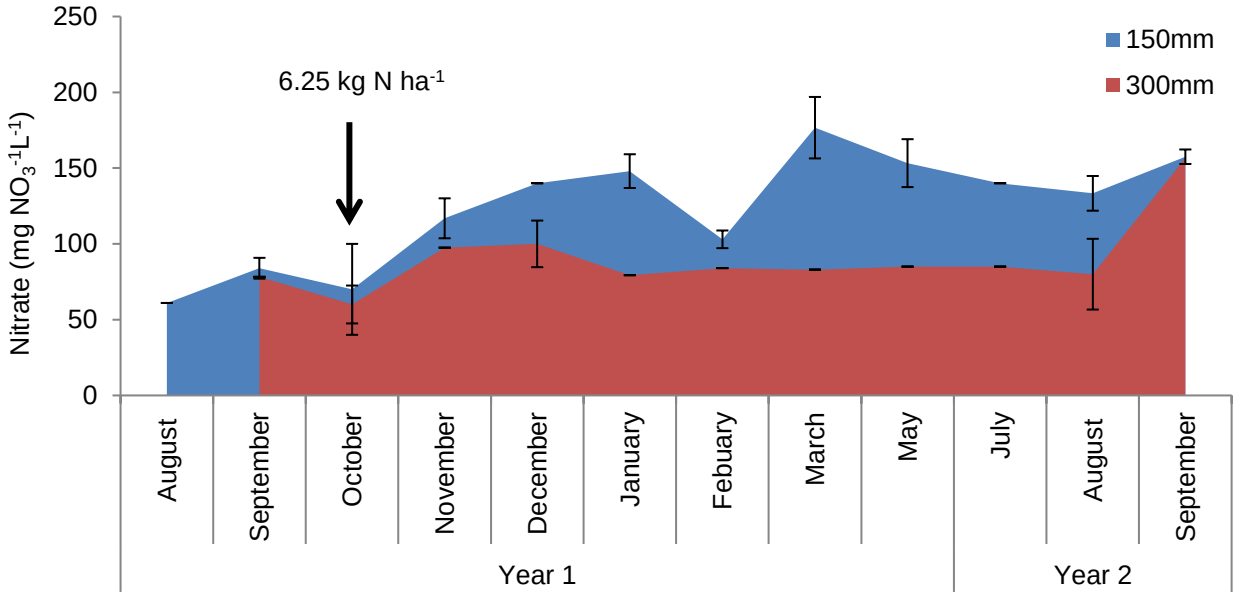

Figure 3.3.1. The average nitrate concentration measured by wetting front detectors at depths of 150 and 300mm respectively. Error bars indicate standard error where more than one observation were recorded. The arrow indicates the average N applied to the four N_{var} plots (6.25 kg N ha^{-1}).

3.3.2 Kikuyu-perennial ryegrass-clover total soil nitrogen

Table 3.3.1 and Figure 3.3.2 show the effect of N fertilisation on total soil N, reported monthly and seasonally, respectively. Total soil N consists out of organic and inorganic soil N. In year one, total soil N was only affected ($P > 0.05$) by N treatments in October and January where N20 displayed the lowest and the highest ($P \leq 0.05$) total soil N, respectively (Table 3.3.1). In July year two, N60 had a higher total soil N than the other treatments. Subsequently, there were no differences ($P > 0.05$) between treatments within the seasons of winter and autumn. However, in spring and summer N20 had the lowest and highest ($P \leq 0.05$) percentage total soil N, respectively (Figure 3.3.2).

The total soil N for all treatments, except N20, decreased ($P \leq 0.05$) from October to November and increased ($P \leq 0.05$) from December to March (Table 3.3.1). This is in agreement with Figure 3.3.2 that illustrates that total soil N for all treatments, except N20, decreased from spring to summer and increased ($P \leq 0.05$) from summer to autumn.

Table 3.3.1. Total soil nitrogen (mg kg^{-1}) to a depth of 100 mm, per month under various fertiliser regimes. Fertiliser regimes: N0, N20, N40, N60 = 0, 20, 40, 60 kg N ha^{-1} applied after grazing; N_{var} = variable nitrogen fertilisation.

		Nitrogen treatment				
Year	**Month**	**N0**	**N20**	**N40**	**N60**	**N_{var}**
1	August	0.210^{a}	0.178^{abcdef}	0.185^{abcd}	0.198^{ab}	0.198^{ab}
	September	0.168^{abcdefg}	0.175^{abcdef}	0.168^{abcdefg}	0.183^{abce}	0.165^{bcdefg}
	October	0.175^{abcdef}	0.115^{hijklmno}	0.175^{abcdef}	0.183^{abcde}	0.173^{abcdefg}
	November	0.105^{jklmno}	0.093^{mo}	0.100^{lmno}	0.093^{no}	0.093^{no}
	December	0.098^{mno}	0.090^{o}	0.088^{o}	0.098^{mno}	0.088^{o}
	January	0.103^{klmno}	0.173^{abcdefg}	0.103^{klmno}	0.110^{hijklmno}	0.103^{klmno}
	February	0.108^{ijklmno}	0.115^{hijklmno}	0.115^{hijklmno}	0.110^{hijklmno}	0.120^{hijklmno}
	March	0.173^{abcdefg}	0.135^{ghijklmn}	0.138^{fghijklm}	0.150^{cdefghi}	0.168^{abcdefg}
	May	0.148^{defghij}	0.130^{ghijklmno}	0.140^{defghijklm}	0.153^{cdefgh}	0.138^{fghijklm}
2	July	0.145^{defghijk}	0.135^{fghijklmn}	0.143^{defghijkl}	0.193^{abc}	0.145^{defghijk}

^{abcde}Means without a common superscript differed significantly (P<0.05).

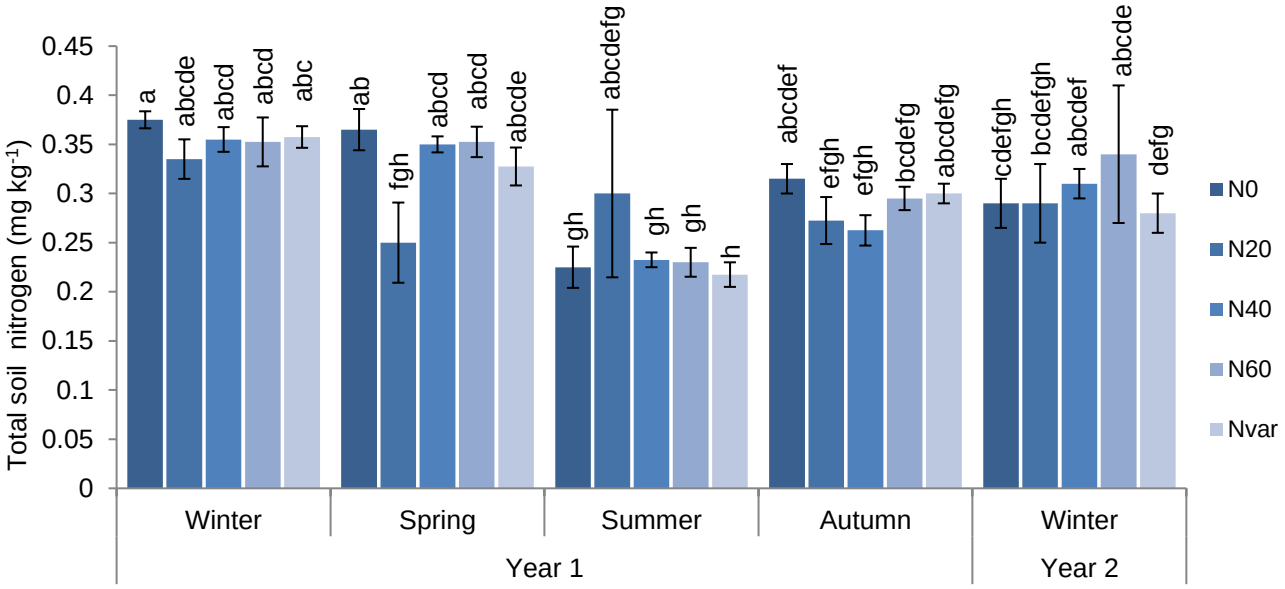

Figure 3.3.2. Total soil nitrogen to a depth of 300 mm per season under various fertiliser regimes. Fertiliser regimes: N0, N20, N40, N60 = 0, 20, 40, 60 kg N ha^{-1} applied after grazing; N_{var} = variable nitrogen fertilisation. Error bars indicate standard error. No common letter above bars denotes a significant difference (P<0.05).

Figure 3.3.3 indicates the relative distribution of total soil N within the top 300 mm soil layer. Treatments were compared within months only. In the top 300 mm of soil, the first 100 mm never contributed less than 45% of the total soil N. For the 0-100 mm depth layer, treatment had no effect on the total soil N distribution except for spring, where N60 and N_{var} displayed a higher N percentage than N20. For both the 100-200 and 200-300 mm depths there were no differences (P > 0.05) between treatments.

The general decrease in total soil N from winter to summer (Table 3.3.1 and Figure 3.3.2) can be accounted for by N losses. According to Ledgard *et al.* (1996) soil N losses can be ascribed to

leaching, denitrification, N volatilisation, and pasture N uptake (Ledgard *et al.* 1996). It was also stated that N losses from leaching, denitrification and volatilisation are much smaller than N lost from pasture uptake. This is supported by Figure 3.3.1 that shows that leaching was relatively constant throughout the trial period for treatment N_{var}. Although leaching was only determined for N_{var}, Figure 3.3.3 shows that soil N distribution fluctuated minimally between treatments at each soil depth. This could indicate that the potential for leaching was minimal for all treatments, and did not change seasonally. However, herbage yield for a KPrCl pasture is expected to be near maximum in summer and minimum in the winter (Botha *et al*. 2008). The significant decrease in total soil N can possibly be ascribed to removal of N through increased pasture growth which in turn might have resulted in increased N uptake. Further investigation of the effects of N removal through pasture growth is warranted. This will be explored further in Chapter 4.

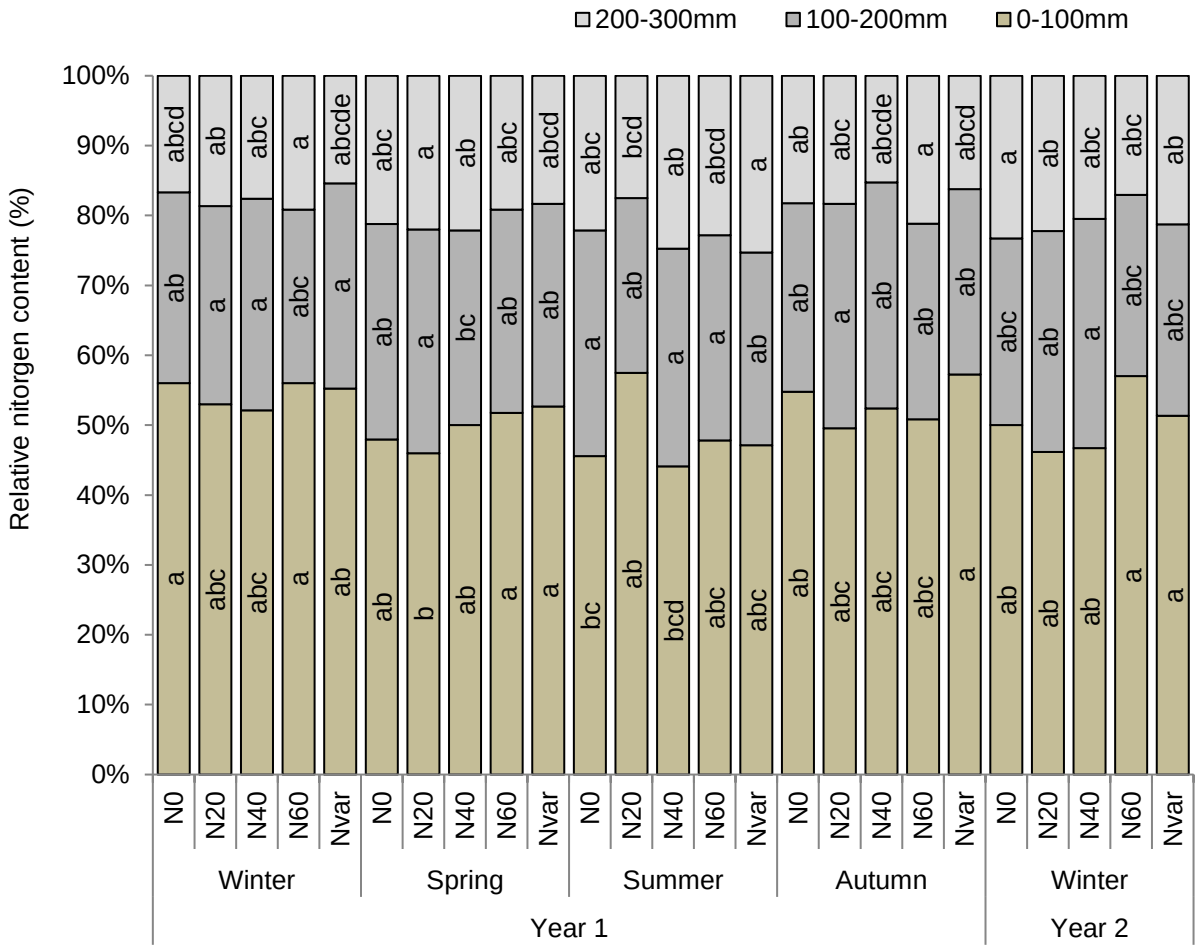

Figure 3.3.3. The percentage contribution of total soil N content at depths of 0-100mm, 100-200mm and 200-300mm. Treatments were compared within season per depth. N0, N20, N40, N60 = 0, 20, 40, 60 kg N ha^{-1}; N_{var} = variable nitrogen fertilisation.

3.3.3 Kikuyu-perennial ryegrass-clover total inorganic soil nitrogen

The total inorganic soil N for all treatments, except N60, remained constant ($P > 0.05$) throughout the study (Figure 3.3.4). Treatment N60, however, had the highest ($P \leq 0.05$) or similar to the

highest total inorganic soil N for the months of October, November, December and March. In March the total inorganic soil N for N60 was particularly high, the reason for this spike in inorganic N is unclear.

Total inorganic soil N increased between September and March under treatment N60. The increase in soil N might suggest that the soil becomes N saturated when N supply surpassed the pastures' N need (Gilliam *et al.* 2005; Zhang *et al.* 2012). The possibility of N saturated soils poses a threat to sustainability. The nitrate ion associated with N saturated soil may facilitate magnesium and calcium leaching, subsequently leading to nutrient imbalances in the soil as well as the plant (Gilliam *et al.* 2005; Zhang *et al.* 2012).

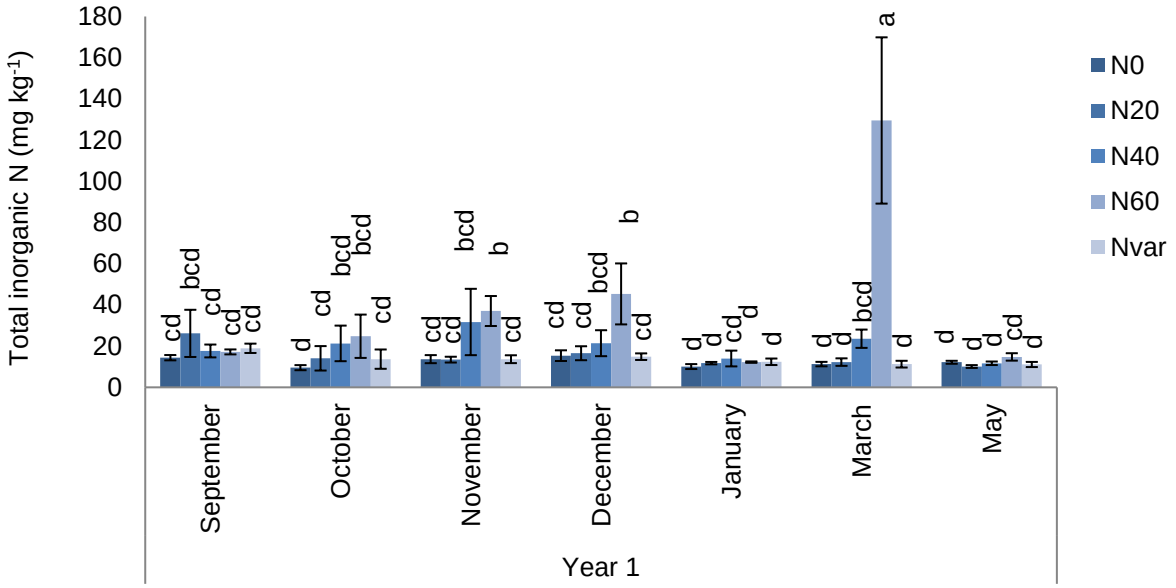

Figure 3.3.4. Total inorganic soil nitrogen to a depth of 100 mm per month under various fertiliser regimes. Fertiliser regimes: N0, N20, N40, N60 = 0, 20, 40, 60 kg N ha^{-1} applied after grazing; N_{var} = variable nitrogen fertilisation. Error bars indicate standard error. No common letter above bars denotes a significant difference ($P<0.05$).

3.3.4 Kikuyu-perennial ryegrass-clover potential mineralisable soil nitrogen

The potential mineralisable N (PMN), measured to a depth of 100 mm, is expressed in Table 3.3.2 as kilogram N released per hectare per grazing cycle. Nitrogen fertilisation treatments had no effect ($P > 0.05$) on PMN within months (Table 3.3.2). The PMN for treatment N40, N60 and N_{var} did, however, decrease ($P \leq 0.05$) from September to May.

Nitrogen mineralisation was reported for most months, however for certain months, immobilisation was observed. Nitrogen immobilisation indicates that N was made unavailable for plant use (Brady and Weil 2002). Nitrogen immobilisation may be accounted for by a decrease in soil temperature or an increase in the soil C/N ratio (Honeycutt *et al.* 1988).

Potential mineralisable N did not follow a fixed trend with regards to N fertilisation treatments. The PMN ranged between 9.5 and 105.9 kg N ha^{-1} grazing $cycle^{-1}$. This coincided with Mishra *et al.* (2005) that reported a gross mineralisation between 4.9 and 135 kg N ha^{-1} grazing $cycle^{-1}$. Hatch *et al.* (2000) reported that under field conditions 50% of N released from gross mineralisation will be immobilised. Assuming that half of the N released from mineralisation is available for plant uptake, mineralised N will vary between 4 and 53 kg N ha^{-1} per grazing cycle.

Table 3.3.2. Potential mineralisable nitrogen (kg N ha^{-1} grazing $cycle^{-1}$) to a depth of 100 mm per month under various fertiliser regimes. Fertiliser regimes: N0, N20, N40, N60 = 0, 20, 40, 60 kg N ha^{-1} applied after grazing; N_{var} = variable nitrogen fertilisation.

		N fertilisation treatment				
Year	**Month**	**N0**	**N20**	**N40**	**N60**	**N_{var}**
1	September	55.7^{abcd}	44.7^{abcde}	105.9^{a}	103.9^{a}	94.8^{ab}
	October	30.5^{bcdef}	41.2^{abcdef}	76.8^{abc}	42.4^{abcdef}	43.7^{abcdef}
	November	32.8^{bcdef}	26.7^{cdef}	46.2^{abcde}	-2.4^{defg}	26.2^{cdef}
	December	17.9^{cdef}	9.5^{cdefg}	-24.1^{fg}	48.9^{abcdef}	17.3^{cdef}
	January	17.9^{cdef}	9.5^{cdefg}	-24.1^{fg}	48.9^{abcdef}	17.3^{cdef}
	March	-10.3^{efg}	-5.5^{defg}	-57.6^{g}	-280.4^{gh}	-6.9^{defg}
	May	-3.6^{defg}	-0.8^{defg}	-5.0^{defg}	-0.8^{defg}	0.0^{defg}

^{abcde}Means without a common superscript differed significantly (P<0.05).

3.3.5 Kikuyu-perennial ryegrass-clover soil urease activity

Nitrogen fertilisation treatments had no effect (P > 0.05) on urease activity within months (Figure 3.3.5). Between months urease activity also did not differ (P > 0.05), except for urease activity of treatment N60 that decreased (P ≤ 0.05) from September to December.

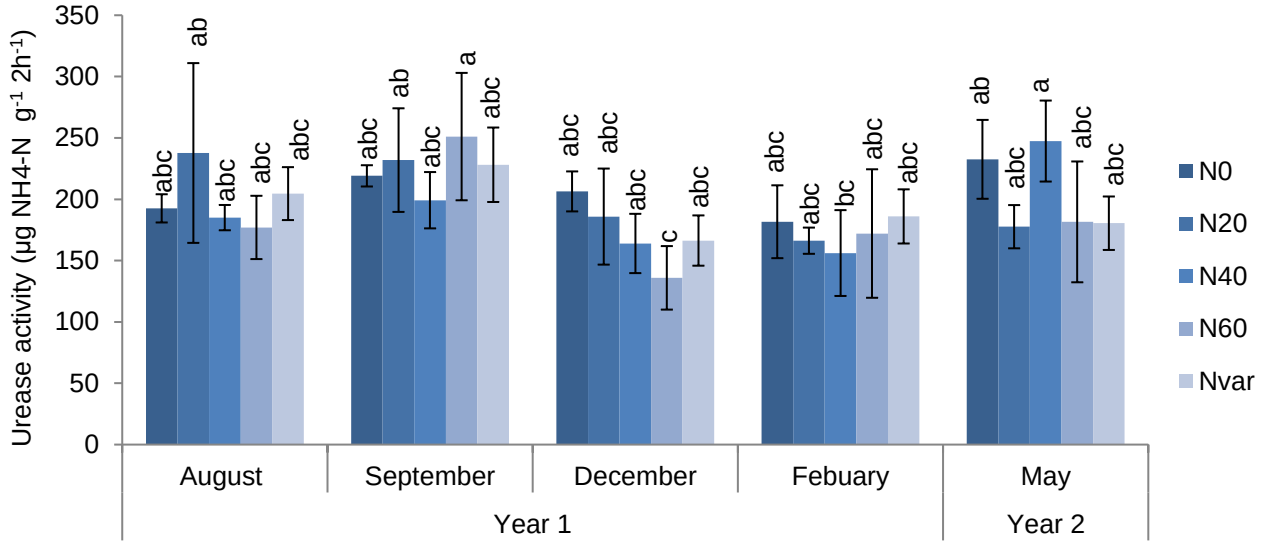

Figure 3.3.5. Urease activity to a depth of 100 mm under various fertiliser regimes. Fertiliser regimes: N0, N20, N40, N60 = 0, 20, 40, 60 kg N ha^{-1} applied after grazing; N_{var} = variable nitrogen fertilisation. Error bars indicate standard error. No common letter above bars denotes a significant difference (P<0.05).

Urease is one of the enzymes responsible for catalysing the process of N mineralisation (Das and Varma 2011). Dick (1992) stated that urease activity can be correlated with N mineralisation, however, as shown in Table 3.3.2, PMN varied minimally between treatments or months. Urease activity may possibly have been related to PMN values. Even though urease did not respond to N fertilisation treatments, the average urease activity found by this study coincided with urease activity rates reported by Swanepoel *et al.* (2017).

3.3.6 Kikuyu-lucerne soil nitrate

As explained in section 3.3.1, irrigation was managed to not exceed a soil water potential of -10 kPa. Water could only be collected form the WFD once soil water potential increased above -3 kPa. Consequently, data collected from the 150 mm depth WFD was insufficient and statistical analyses could not be performed. Figure 3.3.6 shows the average nitrate concentration measured from WFDs planted at 150 mm and 300 mm, respectively. Nitrogen fertilisation is indicated by the black arrow and was applied in October (Figure 3.3.6).

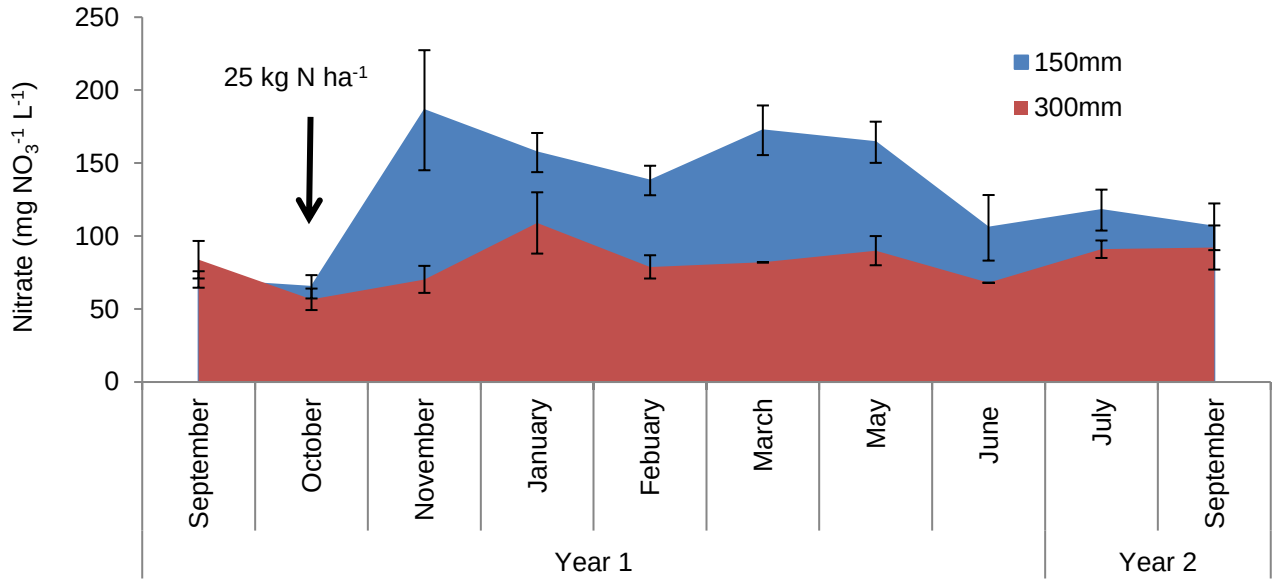

Figure 3.3.6. The average nitrate concentration as measured by wetting front detectors at depths of 150 and 300 mm respectively. Error bars indicate standard error. Arrow indicates the average N applied to the four N_{var} plots (25 kg N ha^{-1}).

For a KL pasture, 60-70% of the roots are concentrated in the first 150 mm of the soil (Ward *et al.* 2002). Although the taproot system of lucerne can penetrate the soil up to 1 m deep, the nitrate measured by the 150 mm WFD shows the nitrate that will largely be available for pasture uptake. Nitrate concentration measured from the 300 mm WFD may therefore not be easily accessible for pasture uptake and can be subjected to leaching. It is expected that the potential leachable N in the KL pasture type will be less than that of the KPrCl pasture type. Nitrate concentration measured from the WFD at a 150 mm depth ranged between 65 mg L^{-1} in October to 186 mg L^{-1} in November (Figure 3.3.6). The nitrate concentration from the 300 mm WFD varied between 57 and

92 mg L^{-1}. The N application made in October might have been the reason for the increase in nitrate recorded for the 150 and 300 mm WFD, from October to November.

In a similar trend to that observed in the KPrCl pasture type, the nitrate concentration measured from the 300 mm WFD always exceeded 50 mg L^{-1}. When the nitrate concentration of water exceeds 50 mg NO_3^- L^{-1}, it is considered unsafe for human intake (Andrews *et al.* 2007). Since nitrate concentration measured at 300 mm is mostly unavailable for pasture uptake it may be subjected to leaching. The leached nitrate might end up in ground water and pose an environmental as well as a health risk.

3.3.7 Kikuyu-lucerne total soil nitrogen

The monthly and seasonal changes in total soil N, in response to N fertilisation, are shown in Table 3.3.3 and Figure 3.3.7 respectively. Treatment had no effect ($P > 0.05$) on monthly as well as seasonally measured total soil N. However, total soil N, for all treatments, decreased ($P \leq 0.05$) from September to January and increased ($P \leq 0.05$) from May to June. The seasonal data also reflects a decrease ($P \leq 0.05$) in total soil N from spring to summer for all treatments, except N_{var}. From spring to winter total soil N for N20 and N40 increased ($P \leq 0.05$).

Table 3.3.3. Total soil nitrogen (mg kg^{-1}) to a depth of 100 mm per month under various fertiliser regimes. Fertiliser regimes: N0, N20, N40, N60 = 0, 20, 40, 60 kg N ha^{-1} applied after grazing; N_{var} = variable nitrogen fertilisation.

		Nitrogen treatment				
Year	**Month**	**N0**	**N20**	**N40**	**N60**	**N_{var}**
Year 1	September	0.168^{abcd}	0.188^{ab}	0.178^{abc}	0.195^{a}	0.168^{abcd}
	October	0.163^{abcde}	$0.145^{cdefghi}$	$0.138^{cdefghijk}$	0.153^{bcdefg}	$0.140^{cdefghij}$
	November	0.100^{klm}	0.098^{lmn}	0.093^{mn}	0.095^{mn}	0.093^{mn}
	January	0.103^{jklmn}	0.090^{mn}	0.100^{klmn}	$0.118^{ghijklm}$	0.110^{ijklmn}
	February	$0.128^{efghijklm}$	$0.125^{efghijklm}$	$0.135^{defghijkl}$	0.106^{jklmn}	$0.113^{hijklmn}$
	March	0.105^{jklmn}	$0.113^{hijklmn}$	0.105^{jklmn}	0.103^{jklmn}	0.110^{ijklmn}
	May	0.108^{ijklmn}	$0.118^{ghijklm}$	0.110^{ijklmn}	$0.123^{fghijklm}$	0.090^{mn}
	June	0.160^{abcdef}	0.153^{bcdefg}	0.168^{abcd}	0.170^{abcd}	$0.150^{bcdefgh}$

^{abcd}Means without a common superscript differed significantly ($P<0.05$).

Figure 3.3.8 indicates the distribution of total soil N within the top 300 mm soil layer. Treatments were compared within month. More than 50% of total soil N in the top 300 mm soil layer was always present in the first 100 mm. For the 0-100 mm layer, N treatment only influenced total soil N distribution in the winter were N0 and N60 was higher than N20. In the 100-200 mm layer, treatment had no effect. For the 200-300 mm layer, treatment only influenced N distribution during autumn, where N percentage was lower under N0 and N60 than N40.

The general decrease in total soil N from winter to summer is a similar trend to that observed with the KPrCl pasture type. With regards to N distribution between soil depths, the KL pasture type was comparable to the KPrCl type and showed that there were minimal differences between treatments (Figure 3.3.8). Likewise, Figure 3.3.6 shows that leaching remained relatively constant throughout the study. Leaching could therefore not have contributed significantly to the decrease in total soil N from winter to summer. Fourie (2015) reported that the herbage production for a KL pasture increases from winter to summer. Therefore, the decrease in total soil N from winter to summer may be attributed to an increase in pasture growth.

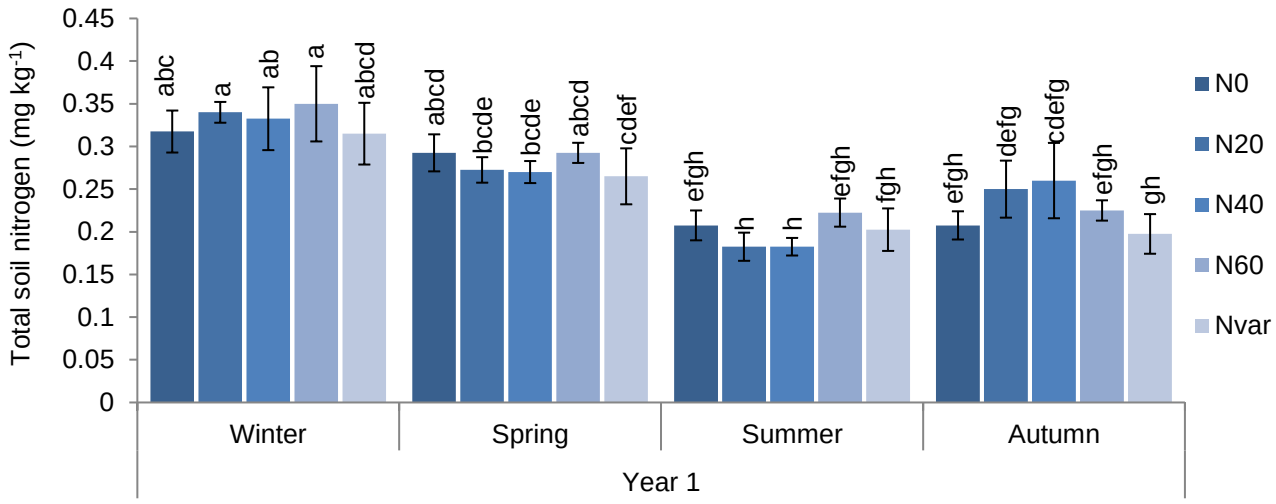

Figure 3.3.7. Total soil nitrogen to a depth of 300 mm per season under various fertiliser regimes. Fertiliser regimes: N0, N20, N40, N60 = 0, 20, 40, 60 kg N ha^{-1} applied after grazing; N_{var} = variable nitrogen fertilisation. Error bars indicate standard error. No common letter above bars denotes a significant difference ($P<0.05$).

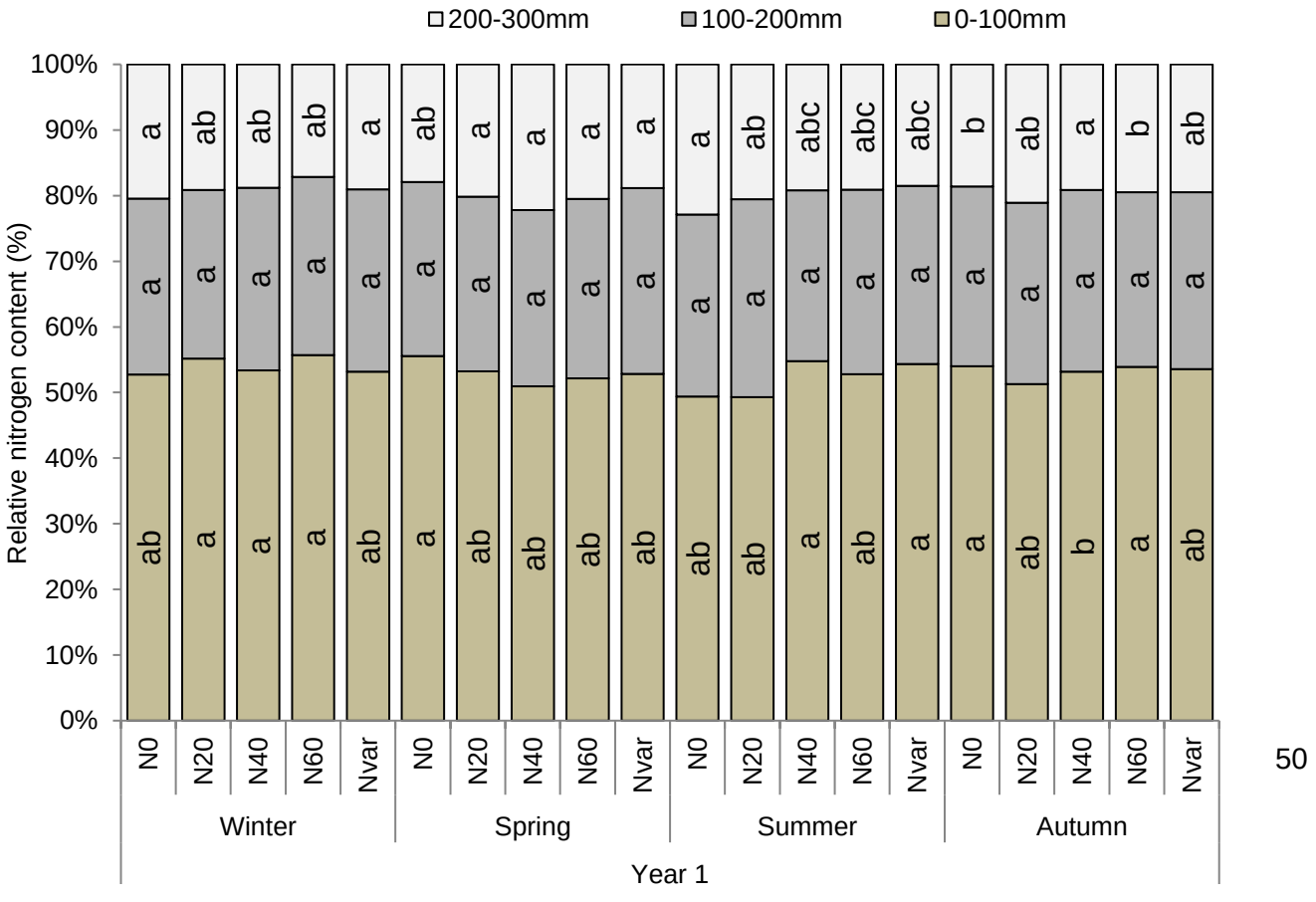

Figure 3.3.8. The percentage contribution of total N content at depths of 0-100mm, 100-200mm and 200-300mm. Treatments were compared within season per depth. N0, N20, N40, N60 = 0, 20, 40, 60 kg N ha^{-1}; N_{var} = variable nitrogen fertilisation.

3.3.8 Kikuyu-lucerne total inorganic soil nitrogen

Total inorganic soil nitrogen did not differ (P > 0.05) between treatments in September (Figure 3.3.9). From September to October total inorganic soil N decreased for all treatments (P ≤ 0.05) except N20. Treatment had no effect (P > 0.05) on total inorganic N from October to November. From November to January total inorganic soil N for all treatments remained constant except for that of N60 which increased (P ≤ 0.05). In January, total inorganic N was the higher (P ≤ 0.05) under N60 than N_{var}. From January to February total inorganic soil N for all treatments remained constant (P > 0.05). In February total inorganic soil N did not differ (P > 0.05) between treatments. From February to March, total inorganic soil N for treatment N20 and N60 increased (P ≤ 0.05), whilst the rest remained constant (P > 0.05). From March to May, the total inorganic soil N for treatment N60 increased (P ≤ 0.05) whilst the rest remained constant (P > 0.05).

Total inorganic soil N under treatment N60 increased between October and March which is similar to results found for the KPrCl pasture type. The increase in inorganic soil N might suggest that N supply under N60 surpassed the pastures' N need (Gilliam *et al.* 2005; Zhang *et al.* 2012).

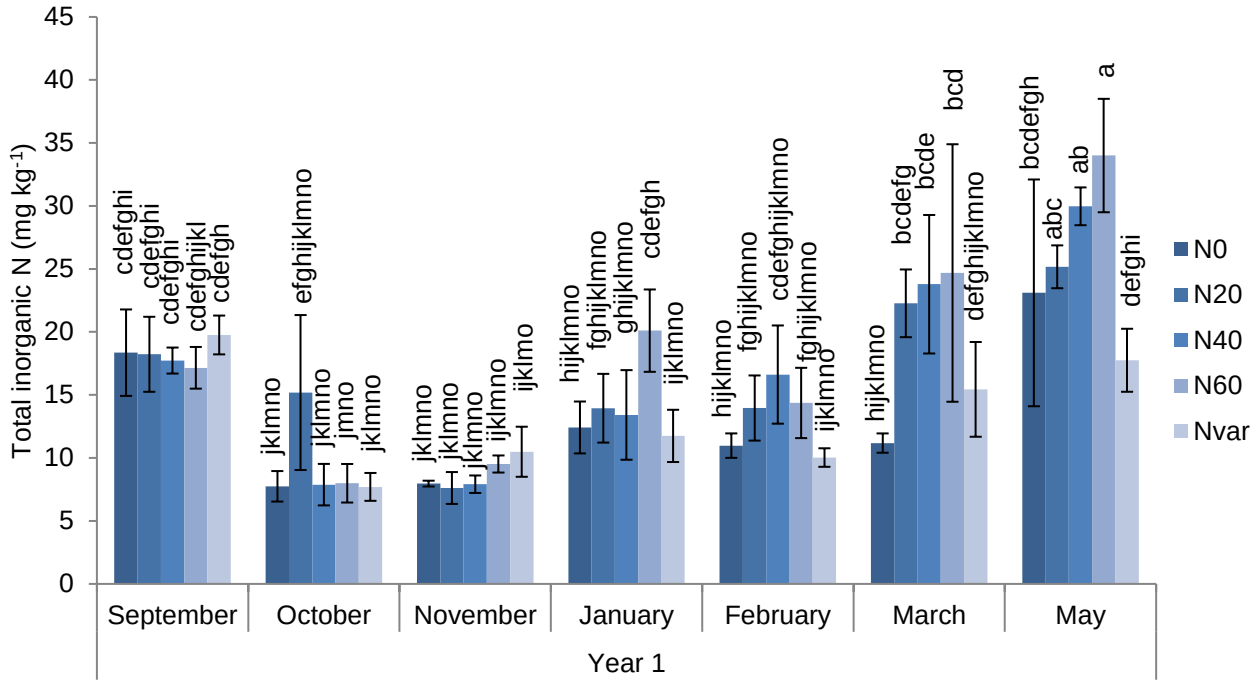

Figure 3.3.9. Total inorganic soil nitrogen to a depth of 100 mm per month under various fertiliser regimes. Fertiliser regimes: N0, N20, N40, N60 = 0, 20, 40, 60 kg N ha^{-1} applied after grazing; N_{var} = variable nitrogen fertilisation. Error bars indicate standard error. No common letter above bars denotes a significant difference (P<0.05).

Kikuyu-lucerne potentially mineralisable soil nitrogen

Table 3.3.4 shows PMN, expressed as kilogram N released per hectare per grazing cycle, measured to a depth of 100 mm. Nitrogen fertilisation treatments had no effect ($P > 0.05$) on PMN within months. The PMN for treatment N20, N40 and N60 did however decrease ($P \leq 0.05$) from September to March.

Similarly to the KPrCl pasture type, PMN for the KL pasture type did not differ between treatments and immobilisation predominantly occurred in March onwards. The PMN varied between 17 and 106.3 kg N ha^{-1} grazing $cycle^{-1}$. The results that were found were in accordance with literature (Mishra *et al.* 2005; Zhang *et al.* 2012).

Table 3.3.4. Potential mineralisable nitrogen (kg N ha^{-1} grazing $cycle^{-1}$) to a depth of 100 mm per month under various fertiliser regimes. Fertiliser regimes: N0, N20, N40, N60 = 0, 20, 40, 60 kg N ha^{-1} applied after grazing; N_{var} = variable nitrogen fertilisation.

		N fertilisation treatment				
Year	**Month**	**N0**	**N20**	**N40**	**N60**	**N_{var}**
1	September	34.9^{bcdefg}	81.5^{abc}	$10.2^{cdefghi}$	30.9^{bcdefg}	50.2^{abcde}
	October	81.3^{abc}	42.1^{abcdef}	$26.8^{bcdefgh}$	106.3^{ab}	58.2^{abcd}
	November	25.5^{cdefgh}	29.1^{bcdefg}	20.1^{cdefg}	$18.9^{cdefghi}$	$17.0^{cdefghi}$
	January	19.7^{cdefgh}	15.8^{cdefgi}	19.9^{cdefgh}	-29^{fghijk}	40.2^{abcdef}
	February	33.1^{bcdefg}	$-25.6^{efghijk}$	$-6.8^{defghij}$	$0.4^{defghij}$	$13.05^{cdefghi}$
	March	26.8^{cdefgh}	-48.7^{hijk}	-49.4^{hijk}	-33.7^{hijk}	$-19^{defghij}$
	May	-40.5^{ghijk}	-58.0^{ijk}	-49.4^{k}	-68.9^{jk}	$-10.1^{defghijk}$

^{abcd}Means without a common superscript differed significantly ($P<0.05$).

3.3.9 Kikuyu-lucerne soil urease activity

There were no differences ($P > 0.05$) in urease activity within months, except in February when that of treatment N40 was equal ($P > 0.05$) to N0 but higher ($P \leq 0.05$) than the rest (Figure 3.3.10). In May N20 was also higher ($P \leq 0.05$) than N60. Between months treatments did not vary ($P > 0.05$).

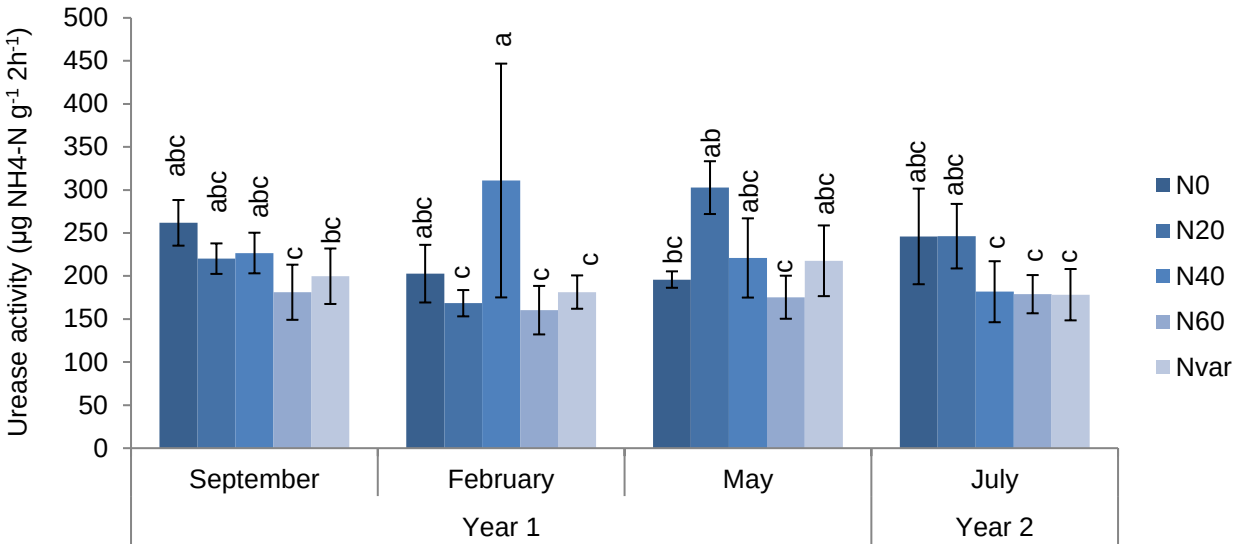

Figure 3.3.10. Urease activity to a depth of 100 mm under various fertiliser regimes. Fertiliser regimes: N0, N20, N40, N60 = 0, 20, 40, 60 kg N ha^{-1} applied after grazing; N_{var} = variable nitrogen fertilisation. Error bars indicate standard error. No common letter above bars denotes a significant difference ($P<0.05$).

The urease activity found for the KL pasture type resembled that found for the KPrCl pasture type. For the KL pasture site, PMN was not influenced by N fertilisation (Table 3.3.4). Therefore, in a similar trend to that of the KPrCl pasture site, the urease activity may be related to PMN. The average urease activity results found for the KL site were also similar to results reported by Swanepoel *et al.* (2017) for kikuyu-ryegrass pasture in the region.

3.4 Conclusions and recommendations

Determining a variable N application with the use of WFDs was unsuccessful in this study. Nitrate concentration could not routinely be measured due to the method employed for irrigation scheduling. Furthermore, the nitrate concentration measured from the 300 mm WFD indicated that N was being leached from the root zone, as the nitrate concentration from the 300 mm WFD was higher than 50 mg L^{-1} throughout the study for both pasture types. Although N was only fertilised once on these experimental units, the high nitrate levels indicate that a significant amount of leaching occurred. Since the N_{var} received minimal amounts of N, it can be concluded that N leaching may have been even higher at experimental units that received higher N rates. Although the use of a WFD could not result in an accurate determination of a variable N rate, soil parameters can be used. Total soil N decreased from winter to summer for all treatments and both pasture types. This can potentially be ascribed to an increase in yield for the same period and thus an increase in the pastures' N uptake. It can thus be deduced that N fertilisation rate has to fulfil the pastures' N need, which in turn is likely to be determined by seasonal production rates. The N application rate for kikuyu-legume pastures in the southern Cape should increase from winter to summer. Potential mineralisable N was not influenced by N fertilisation treatment. However, in certain seasons, the N released from mineralisation equated to more than 100 kg N ha^{-1} grazing $cycle^{-1}$. The N released by mineralisation must be accounted for by a variable N fertilisation regime. Urease activity did not respond to N fertilisation regimes. This may be due to the fact that the high

N level in the soil surpasses the soil microbes' capacity. Only one soil parameter, total inorganic soil N, was significantly affected by N fertilisation rate. Total inorganic soil N increased under the N60 fertilisation treatment for both pasture types throughout the study. This indicates that the soil becomes saturated with N under the N60 fertilisation regime and that a variable N fertilisation rate should not exceed N60. Total inorganic soil N was the parameter that was the most sensitive to N treatments and therefore should be used in future studies instead of urease activity.

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CHAPTER 4

Nitrogen fertilisation effects on herbage yield, quality and botanical composition of kikuyu-based pastures over-sown with ryegrass and legumes

4.1 Introduction

Kikuyu (*Pennisetum clandestinum*), is an important species in the cultivated pastures in the dairy producing region of the southern Cape (Van der Colf *et al.* 2015a). This grass species produces high herbage yields and can sustain high stocking rates from summer to autumn (Reeves 1997; Botha *et al.* 2008a; Botha *et al*. 2008b). Kikuyu becomes dormant as atmospheric temperatures drop below 10ºC and consequently herbage production declines from autumn to spring (Minson *et al*. 1993; Marais 2001). To overcome the gap in fodder flow during this period, kikuyu is generally over-sown by ryegrass (*Lolium spp*.) in autumn (Botha et al. 2008a; Van der Colf *et al*. 2015b). A kikuyu-ryegrass pasture can yield up to c. 21 t dry matter (DM) ha^{-1} yr^{-1} (Swanepoel *et al.* 2014). The recommended N fertilisation rate for a kikuyu-ryegrass pasture for this region can be as high as 600 kg N ha^{-1} yr^{-1} (Botha *et al*. 2008a; Swanepoel *et al*. 2015). Legumes can improve pasture quality and have the potential to lower N fertilisation inputs by means of biological N fixation (BNF) (Ledgard and Steele 1992; Andrews *et al.* 2007). Legumes have consequently been included in kikuyu-based pasture systems. Legumes, like lucerne (*Medicago sativa*) and clover (*Trifolium* spp.), can produce yields of 18 and 15.7 t DM ha^{-1} yr^{-1}, respectively, when over-sown into kikuyu (Botha *et al.* 2008a; Fourie 2015). Research conducted in New Zealand determined that a temperate grass-legume pasture can fix up to 250 kg N ha^{-1} yr^{-1} in the absence of N fertilisation (Ledgard and Steele 1992). However, the above-mentioned pastures were found to be N deficient and yielded 25 to 35% less herbage than pastures that received N (Harris *et al*. 1996; Ledgard *et al*. 2001). High inorganic N inputs result in grass out-competing the legumes for light, water and nutrients (Ledgard and Steele 1992; Ledgard 2001). This consequently leads to grass dominating the pasture. In contrast to high N inputs, low inorganic N inputs result in legumes having the competitive advantage (Ledgard and Steele 1992; Ledgard 2001). Harris *et al*. (1996) determined that the clover component of a ryegrass-clover pasture decreased by two-fold when N fertilisation increased from 0 to 400 kg N ha^{-1} yr^{-1}. Findings from this study also showed that legume plant densities decreased as N rates increased. In addition, in comparison to low N fertilisation rates, legumes developed fewer stolons and auxiliary buds when high N fertilisation rates were applied (Harris *et al.* 1996).

Nitrogen fertilisation regimes of grass-legume pasture must therefore take into account the benefits of N fertilisation, while reducing the negative effects of high N inputs on the legume component (Clark and Harris 1996). Guidelines based on recent research for N fertilisation of grass-legumes

pastures in the southern Cape are not currently available. The aim of this study is thus to quantify the effect of different N fertilisation regimes of kikuyu-lucerne and kikuyu-perennial ryegrass-clover pastures in terms of herbage yield, botanical composition and herbage crude protein content.

4.2 Materials and methods

4.2.1 Experimental site

The Outeniqua Research Farm, located in the Western Cape Province of South Africa (33°58'38" S and 22°25'16" E), was used as research site. The region is characterised by a temperate climate with a mean annual rainfall of 728 mm per annum that is distributed throughout the year. Summer is from December to February, with average daily temperatures ranging between 13 and 25ºC (ARC-ISCW 2016). Winter is from June to August, with average daily temperatures varying between 7 and 19ºC (ARC-ISCW 2016).

The experimental area consisted of 1.8 ha of existing minimum-tillage kikuyu pastures under permanent over-head sprinkler irrigation. The area was divided into two separate sites with a pasture type allocated to each site. Pasture types are described in Table 4.2.1. The KPrCl and KL pasture types were managed differently in terms of grazing and irrigation (see section 4.2.4 and 4.2.5). Consequently, the two pasture types were not compared.

Table 4.2.1. Pasture type, over-sown species and cultivars used in this study.

Pasture type abbreviation	Species over sown into kikuyu (*Pennisetum clandestinum*)	Cultivar
KL	Lucerne *(Medicago sativa)*	WL 375
KPrCl	Perrenial ryegrass *(Lolium perenne)*	Arrow
	Red clover *(Trifolium pratense)*	Borduro
	White clover *(Trifolium repens)*	Storm

The study was conducted on a Podzolic soil according to IUSS Working Group (2006) and a Spodosol according to Soil Survey Staff (2003). According to the South African soil classification system, the soil at the site is classified as a Witfontein soil form (Soil Classification Working Group 1991) and is characterised by three diagnostic horizons namely: Orthic A horizon (0 to 350mm), Podzol B horizon (350 to 500mm) and an underlying unconsolidated horizon with signs of wetness (500 to 800mm).

4.2.2 Experimental treatments and layout

4.2.2.1 Treatments

Each of the two trials were laid out as a randomised block design, with five different N fertilisation treatments and four replicates per treatment per site (20 experimental units per site). Plots encompassed 450 m^2 and the trial area was 1.8 ha in total.

Nitrogen fertilisation treatments included fixed rates ranging from 0 to 60 kg N ha^{-1} and a variable rate referred to as a variable N application (N_{var}) (Table 4.2.2). Nitrogen, in the form of limestone ammonium nitrate (LAN), was applied to the experimental units after every grazing event. Nitrogen fertilisation was done by hand. Table 4.2.3 and Table 4.2.4 show the actual amounts of N fertilised per season for both pasture types, respectively. For the KPrCl pasture type (Table 4.2.3), grazing cycles in the autumn and winter were shorter than in the spring and summer. In the cooler months pasture growth is lower and pastures were grazed less frequently. In the autumn of year two, the pasture was over-sown with ryegrass and clover and therefore there was only one grazing cycle. For the KL pasture type (Table 4.2.4), grazing cycles were more frequent in the summer than in the other seasons.

Table 4.2.2. Nitrogen input for fixed nitrogen fertilisation treatments.

Nitrogen fertilisation treatment	**Nitrogen applied after each grazing (kg N ha^{-1})**
N0	0
N20	20
N40	40
N60	60
N_{var}	0, 25 or 50

The variable N application rate was determined by measuring soil solution nitrate from water collected by wetting front detectors (WFD) planted at 150 and 300 mm depths on each N_{var} plot (Fessehazion *et al.* 2011). The WFD is a robust funnel shaped apparatus that was originally designed for small scale farmers as an irrigation scheduling instrument (Stirzaker 2003). The WFD is “planted” or installed in the soil in order to intercept soil water and indicate the percolation depth of water in the soil profile. As water percolates through the soil, it is caught by the wider end of the funnel. If the water continues to move downward, water accumulates in the narrow end of the funnel. Water will accumulate in the funnel until soil matrix potential reaches -3 kPa, after which free water is caught in a reservoir at the base of the WFD. It is accepted that the soil matrix

potential inside the funnel is the same as outside. Floats present in a tube connected to the reservoir are activated when the reservoir is full and an above ground flag is launched. Free water from the reservoir can be extracted with a syringe (Stirzaker 2003). During the study the nitrate concentration of the extracted water was measured with the use of nitrate strips and a LAQUAtwin water quality meter. The N_{var} application rates were based on the average nitrate concentration measured for the previous growth cycle from the WFDs planted at the 150 mm depth.

Table 4.2.3. Actual amounts of N fertilised, for the kikuyu-perennial ryegrass-clover pasture type, per season for each treatment in kg N ha^{-1}.

N treatment	**Season (year)**					
	Winter (1)	**Spring (1)**	**Summer (1)**	**Autumn (1)**	**Winter (2)**	**Spring (2)**
N0	0	0	0	0	0	0
N20	40	60	60	20	40	60
N40	80	120	120	40	80	120
N60	120	180	180	60	120	180
N_{var}	0	6.25	0	0	0	0

Table 4.2.4. Actual amounts of N fertilised, for the kikuyu-lucerne pasture type, per season for each treatment in kg N ha^{-1}.

N treatment	**Season (year)**					
	Winter (1)	**Spring (1)**	**Summer (1)**	**Autumn (1)**	**Winter (2)**	**Spring (2)**
N0	0	0	0	0	0	0
N20	40	40	60	40	40	40
N40	80	80	120	80	80	80
N60	120	120	180	120	120	120
N_{var}	0	25	0	0	0	0

Findings and recommendations originating from research conducted by Fessehazion *et al*. (2011) were used to determine N_{var} application rates from June 2016 to October 2016. However, no N was

applied for this time period which resulted in poor pasture production. As result, N_{var} guidelines were adapted from October 2016 (each interval was increased with 25 mg L^{-1}). Table 4.2.5 summarises the guidelines used to determine N_{var} rates.

Table 4.2.5. Guidelines used to determine N_{var} rate from nitrate concentration measured.

Nitrate concentration intervals (mg L^{-1})	**N_{var} rate applied (kg ha^{-1} N)**
Less than 50	50
Between 50 and 75	25
More than 75	0

4.2.3 Seed treatment and establishment

Fourteen days before planting, the lucerne and clover seeds were treated with a pesticide and fungicide (Table 4.2.6). Mefanoxam protects seeds against *Phythopthora* and *Pythium*, while Dimethoate protects against lucerne flea (*Sminthuris viridis*) and black sand mite (*Halotydeus destructor*) (Smit 1964; Langenhoven 1986; Botha 2003). The clover and lucerne seeds were inoculated on the day of planting with host-specific *Rhizobium* (Ledgard and Steele 1992).

Prior to establishment, the pasture sites were grazed by Jersey cows to a height of 50 mm above ground level. After grazing a Nobili mulcher (1.6 m with 24 blades) was used to pulverise aboveground herbage to ground level (Botha *et al*. 2008a). The different pasture types were established by over-sowing the pasture species into the mulched herbage during April 2016. The species were planted by a 3316 C seedmatic Aitchison no-till seeder with 16 rows. The entire area was rolled with a teff roller after planting to improve seed-soil contact. Lucerne (cv. WL 375) was over-sown at 15 kg ha^{-1} to establish the KL pasture type. Perennial ryegrass cv. Arrow, red clover cv. Borduro and white clover cv. Storm were over-sown at 12, 4 and 4 kg ha^{-1}, respectively to establish the KPrCl pasture type. During April 2017 the KPrCl pasture type was over-sown for a second time by mulching slightly above ground level and planting the same mixture as in year 1. The KL pasture type was not over-sown in year 2. The choice of cultivars and seeding rates were based on previous research findings (Botha *et al*. 2008a; Fourie 2015).

Table 4.2.6. The pesticide type, active ingredient and dosage rate used to treat leguminous seeds before planting.

Pesticide type	Active ingredient (commercial product used)	Dosage rate
Fungicide	Mefenoxam (Apron XL)	50 ml 100 kg^{-1} seed
Insecticide	Dimethoate (Dimethoate)	600 ml 100 kg^{-1} seed (diluted with 4 L of water)

4.2.4 Grazing management

Disc pasture meter readings were taken before and after each grazing event to measure pasture height. Pasture height and density was used to estimate the herbage available to the cows (Stockdale 1984). The estimated herbage available to cows was used to determine the number of Jersey cows needed to graze the pasture to 50 mm above ground level (Van der Colf 2011). The disc pasture meter readings, taken after each grazing, indicated that pastures were always grazed down equally to a height of approximately 50 mm (results not shown). The pastures were strip grazed approximately every 35 days for KL pasture type and approximately every 28 days for the KPrCl pasture type (Botha 2003; Botha *et al*. 2008a; Fourie 2015).

4.2.5 Irrigation management

A permanent overhead sprinkler irrigation system, with sprinklers in a staggered configuration and 15 m spacing between each sprinkler, was used to irrigate pastures. Additionally, the amount of rain water that fell on the trial area was determined by installing a manual rain gauge at each pasture site. Irrigation was scheduled according to the guidelines of Botha (2002), with the use of tensiometers, an apparatus that measures soil matric potential. Tensiometers were installed at the KL and KPrCl pasture sites at 200 mm and 150 mm depths, respectively. Soil matric potential was maintained between -25 kPa and -10 kPa.

4.2.6 Herbage yield and herbage protein

Herbage yield (kg DM ha^{-1}) was estimated monthly by cutting pasture samples within 0.0985 m^2 rings before grazing. Six rings were randomly placed within each plot and cut to a height of 50 mm. Each sample was oven dried at 60^{o}C for 72 hours and weighed to determine herbage yield.

The dried herbage samples used to determine herbage yield were milled and analysed for total herbage N using the Kjeldahl analysis. Crude protein was determined by multiplying the herbage N content by 6.25 (AOAC 2000).

4.2.7 Botanical composition

Seasonal botanical composition was determined by cutting herbage samples to a height of 50 mm above ground level prior to grazing, from three 0.0985 m^2 rings randomly placed within each plot. The herbage from the KPrCl pasture type was fractioned into: clover, perennial ryegrass, kikuyu, other grass species and weeds. Other grasses were classified as grasses that were neither over-sown into the sward or kikuyu, and could influence milk production. These species included: *Poa annua*, *Sporobolus africana, Paspalum notatum, Bromus* spp. and *Eragrostis plana*. Weeds were classified as species that would negatively influence milk or pasture production by either being unpalatable, toxic or harmful to livestock. Weeds species included: *Urtica* spp., *Cyperus* spp., *Arctotheca calendula, Taraxacum officinale, Stellaria media* and *Rumex crispus*. The herbage from the KL pasture type was separated into five fractions, namely: lucerne, kikuyu, grass species other than kikuyu (other grass), legumes other than lucerne (other legumes) and weeds. The other grasses and weeds component were classified similarly to the KPrCl pasture site. Other legumes mostly included *Trifolium* spp. Each fraction was dried for 72 hours at 60°C to determine the fractions' contribution on a DM basis (Fenthum-Vermeulen 2009).

4.2.8 Statistical analyses

The Restricted Maximum Likelihood (REML) procedure was followed using Variance Estimation, Precision and Comparison (VEPAC) package of STATISTICA (Dell Inc. 2016). The fixed effects were treatment, date and the interaction between treatment and date. Compensation for random effects included effects of block, the cross between block and treatment and the cross between block and date. Normality of residuals were tested for constant treatment variables. The treatment means were compared by carrying out a student t-test at a 5% significance level.

4.3 Results and discussions

4.3.1 Kikuyu-perennial ryegrass-clover herbage production

The monthly herbage yield (kg DM ha^{-1}) of kikuyu-perennial ryegrass-clover, in response to N fertilisation regimes, is shown in Table 4.3.1. For year one, the herbage yield of treatment N60 was higher ($P \leq 0.05$) than for N_{var} from August to October when compared within months. From November to January there were no differences ($P > 0.05$) between treatments. During February and March N60 had a higher ($P \leq 0.05$) yield than N_{var}. The yield for treatment N60 and N40 was higher ($P \leq 0.05$) than N0 in May (year 1) and July (year 2). In August of year two, the yield of N60 was higher ($P \leq 0.05$) than N0. From September until November, treatment had no effect ($P > 0.05$) on yield.

In terms of seasonal yield, treatment had no effect ($P > 0.05$) on yield in the winter, summer and autumn of year one as well as the winter and spring of year two (Table 4.3.2). In the spring of year one, however, N60 produced a yield of 7033 kg DM ha^{-1}, which was higher ($P \leq 0.05$) than for N_{var}

(6027 kg DM ha^{-1}). The herbage yield for all treatments increased ($P \leq 0.05$) from winter to spring, remained constant ($P > 0.05$) from spring to summer and decreased ($P \leq 0.05$) from summer to autumn in year one. From autumn in year one to winter in year two the yield for all treatments, except N60 decreased ($P \leq 0.05$). The yield for all treatments increased ($P \leq 0.05$) from winter in year two, to spring in year two.

Although treatment had an effect on herbage yield in certain months, the total annual yield was not affected ($P > 0.05$) by N fertilisation treatments (Figure 4.3.1). This is in contrast with N fertilisation studies that were conducted on ryegrass-clover pastures in New-Zealand (Clark and Harris 1996; Shiel *et al.* 1999; Ledgard *et al.* 2001). Ledgard *et al.* (2001) reported yields of 16.4, 18.5 and 20.6 t DM ha^{-1} yr^{-1} under N fertilisation regimes of 0, 200 and 400 kg N ha^{-1} yr^{-1}, respectively. The yields reported under the 400 kg N ha^{-1} yr^{-1} fertilisation regimes are similar to the average annual yield (20.4 t DM ha^{-1} yr^{-1}) found by this study. The yields reported under 0 and 200 kg N ha^{-1} yr^{-1} were lower than the annual average yield found by this study. The lower yields may possibly be attributed to a difference in location and rainfall. The long-term average rainfall reported by Ledgard *et al*. (2001) was 1200 mm yr^{-1}. This is 475 mm yr^{-1} more than that found for this study's trial site. The higher rainfall may have led to increased soil N losses through leaching and volatilisation (Ledgard *et al.* 1996). Therefore, less N could have been available for pasture uptake under the 0 and 200 kg N ha^{-1} yr^{-1} regime in comparison to the 400 kg N ha^{-1} yr^{-1} regime. These studies that took place in New-Zealand also did not include a kikuyu component.

Botha *et al.* (2008) measured the yield of a kikuyu-perennial ryegrass-clover pasture in the vicinity of the current study, which received no N. They reported an annual yield of 15.9 t DM ha^{-1} yr^{-1}. However, the reported yield is c. 3 t DM ha^{-1} yr^{-1} less than that found under treatment N0 and N_{var}. This difference may be explained by the different clover and ryegrass cultivars that were used. Additionally, grazing management as well as herbage measurement may have contributed to yield differences. When pastures are non-uniformly grazed, herbage may be carried over from one grazing cycle to the next. This will result in inaccurate calculation of yield. Botha *et al.* (2008) made use of the rising plate meter and calibration curves to determine yield. This technique may have resulted in more accurate yield estimation.

Although N from cow excreta was not measured, it could have potentially influenced herbage yield. Ledgard (2001) stated that a single urine patch may equate to a localised input of 1000 kg N ha^{-1}. Ledgard *et al*. (1996) determined that when cows are fed from pasture, excreta-N increases with N fertilisation rate. Cows will therefore return more N to the pasture when pastures are fertilised with high N rates in comparison to a pasture that received low N rates. However, the experimental site used for this study was strip grazed and cows moved freely between experimental units. A carry-over of N, through excreta, might have occurred between experimental units. As result of these grazing management practices and the experimental design, excreta-N was theoretically

distributed equally throughout the trial area. This may have reduced the effect of the N treatments. When N fertilisation regimes are determined for grass-legume pastures, the N concentration of excreta should be accounted for.

Table 4.3.1. The total monthly herbage yield (kg DM ha^{-1}) of a kikuyu-perennial ryegrass-clover pasture in response to nitrogen fertilisation treatments applied after grazing. N0, N20, N40, N60 = 0, 20, 40, 60 kg N ha-1; N_{var} =variable nitrogen fertilisation. Treatments were compared within months. No common denotes a significant difference (P<0.05)

		Nitrogen treatment				
Year	**Month**	**N0**	**N20**	**N40**	**N60**	**N_{var}**
1	August	1735^{d}	1787^{cd}	2196^{ab}	2154^{b}	1677^{d}
	September	2371^{abc}	2305^{bc}	2587^{ab}	2720^{a}	2187^{c}
	October	1837^{c}	1891^{c}	2008^{bc}	2120^{ab}	1884^{c}
	November	1962^{c}	2119^{bc}	2190^{abc}	2191^{bc}	1955^{c}
	December	1985^{abcd}	1859^{bcd}	1824^{cd}	1866^{bcd}	1817^{d}
	January	2723^{abc}	2494^{c}	2582^{bc}	2558^{bc}	2543^{c}
	February	2019de	2183^{bcde}	2130^{cde}	2407^{abc}	1901^{e}
	March	1389de	1610^{abcd}	1467^{cde}	1586^{bcd}	1205^{e}
	May	1354^{e}	1570^{cde}	1723^{abcd}	1659^{bcde}	1452de
2	July	918^{d}	1140^{bcd}	1181^{bcd}	1282^{abc}	1036^{cd}
	August	824^{bc}	1035^{ab}	1011^{ab}	1255^{a}	865^{abc}
	September	1226^{ab}	1186^{abc}	1247^{ab}	1392^{a}	1081^{abcd}
	October	1613^{ab}	1726^{a}	1563^{abc}	1748^{a}	1453^{abcd}
	November	1916^{a}	1791^{ab}	1927^{a}	1924^{a}	1780^{ab}

^{abcd}Means without a common superscript differed significantly (P<0.05).

Table 4.3.2. The total seasonal herbage yield (kg DM ha^{-1}) of a kikuyu-perennial ryegrass-clover pasture in response to nitrogen fertilisation treatments applied after each grazing. N0, N20, N40, N60=0, 20, 40, 60 kg N ha^{-1}; N_{var}=variable nitrogen fertilisation.

	Nitrogen treatment				
Season	**N0**	**N20**	**N40**	**N60**	**N_{var}**
Winter 1	4156^{d}	4208^{cd}	4480^{cd}	4575^{cd}	4098de
Spring 1	6172^{ab}	6317^{ab}	6787^{ab}	7033^{a}	6027^{b}
Summer 1	6728^{ab}	6537^{ab}	6538^{ab}	6832^{ab}	6263^{ab}
Autumn 1	2743^{fgh}	3181^{f}	3191^{f}	3245^{ef}	2658^{fg}
Winter 2	1743^{i}	2175^{ghi}	2192^{ghi}	2538^{fghi}	1901^{hi}
Spring 2	4755^{cd}	4703^{cd}	4738^{cd}	5064^{c}	4315^{cd}

^{abcd}Means without a common superscript differed significantly (P<0.05).

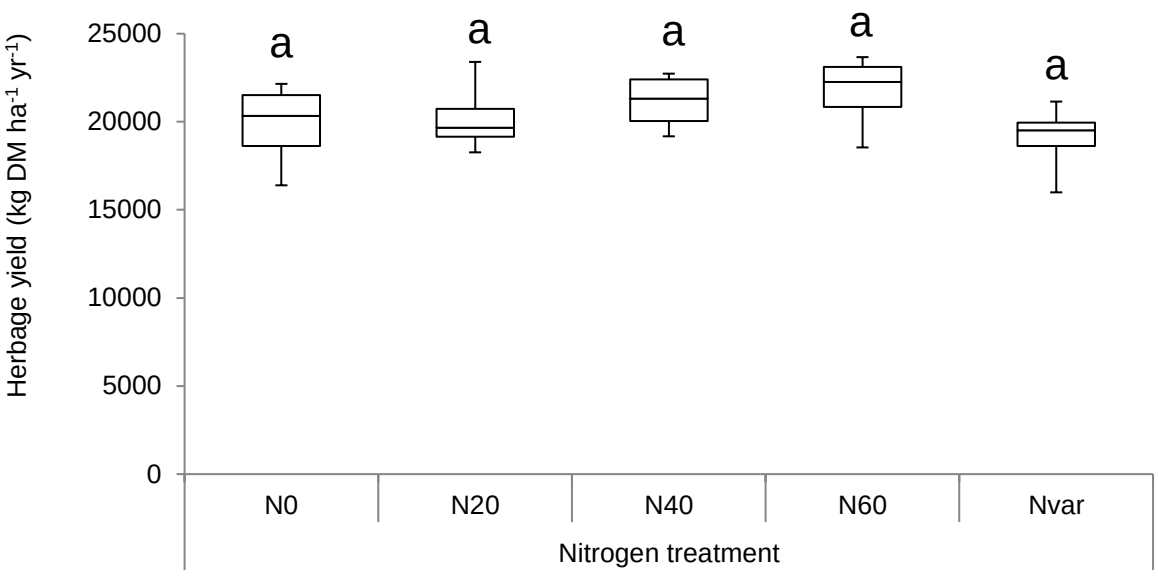

Figure 4.3.1. The total annual herbage yield (kg DM ha^{-1} yr^{-1}) of a kikuyu-perennial ryegrass-clover pasture, during year one, in response to nitrogen fertilisation treatments applied after grazing. N0, N20, N40, N60 = 0, 20, 40, 60 kg N ha^{-1}; N_{var} =variable nitrogen fertilisation. No common letter above bars denotes a significant difference (P<0.05).

4.3.2 Kikuyu-perennial ryegrass-clover botanical composition

The contribution of kikuyu, ryegrass and clover to botanical composition is shown in Figure 4.3.2, Figure 4.3.3 and Figure 4.3.4 respectively. For year one, the kikuyu component for all treatments remained constant ($P > 0.05$) from winter to spring except for N40, which decreased ($P \leq 0.05$) (Figure 4.3.2). In spring 1 there were no differences ($P > 0.05$) between treatments. From spring to summer, the kikuyu component for N20, N40 and N60 increased ($P \leq 0.05$), while N0 and N_{var} remained constant ($P > 0.05$). In summer there were no differences ($P > 0.05$) between treatments in terms of kikuyu content. From summer to autumn in year one the kikuyu component for N20, N40 and N60 increased ($P \leq 0.05$), while the rest remained constant ($P > 0.05$). From autumn in year one to winter in year two, the kikuyu component for treatment N20, N40 and N60 decreased ($P \leq 0.05$), while N0 and N_{var} remained constant ($P > 0.05$). From winter to spring in year 2, the kikuyu component for N40 and N_{var} decreased ($P \leq 0.05$). In spring there were no differences ($P > 0.05$) between treatments.

The ryegrass component of treatment N0, N40 an N60 increased from winter to spring, while that of N20 and N_{var} decreased ($P \leq 0.05$) (Figure 4.3.3). In spring, N40 had an equal ($P > 0.05$) ryegrass component to N60 but higher ($P \leq 0.05$) than the rest. The ryegrass component under all treatments decreased ($P \leq 0.05$) from spring to summer. In summer N40 and N60 had a similar ($P > 0.05$) ryegrass content, but higher ($P \leq 0.05$) than the rest. From summer to autumn, the ryegrass component of N40 and N60 decreased ($P \leq 0.05$), while the rest did not differ ($P > 0.05$). In autumn there were no differences ($P > 0.05$) between treatments. From autumn in year one to winter in year two, the ryegrass component for all treatments increased. This was likely associated with the over-sowing of ryegrass during the second autumn. In autumn, N60 had a higher ($P \leq$

0.05) ryegrass component than N0 and N_{var}. From winter to spring the ryegrass component for all treatments remained constant ($P > 0.05$). In the spring, N20, N40 and N60 had a higher ryegrass content ($P \leq 0.05$) than N0 and N_{var}.

For year one, the clover component for all treatments remained constant ($P > 0.05$) throughout seasons, except for N20 and N_{var}, where it increased ($P \leq 0.05$) from winter to spring (Figure 4.3.4). In winter, treatment N0 had a higher ($P \leq 0.05$) clover component than the rest. In spring N0 had a higher clover component than N60 ($P \leq 0.05$). In the summer N60 and N40 had lower clover contents ($P \leq 0.05$) than N0 and N_{var}. In autumn the clover content of N_{var} was higher ($P \leq 0.05$) than N20 but similar ($P > 0.05$) to the rest. In year two, there were no differences between treatments in the winter. From winter to spring the clover component of N0 and N_{var} increased ($P \leq 0.05$). In the spring N0 and N_{var} had an equal ($P > 0.05$) clover component but higher ($P \leq 0.05$) the other.

As shown in Figure 4.3.2 to Figure 4.3.4, the grass components tended to respond positively to N fertilisation, while the clover component tended to decrease under the highest N fertilisation regime. Shiel *et al.* (1999) reported, for a ryegrass-clover pasture, that clover content decreased by more than 50% when N rates increased from 0 to 400 kg ha^{-1} yr^{-1}. They additionally determined that ryegrass production was positively related to N fertilisation rates. Harris *et al.* (1996) determined that the clover content, of a ryegrass-clover pasture, decreased by 45% when N fertilisation increased from 0 to 400 kg ha^{-1} yr^{-1}. Similarly, Ledgard and Steele (1992) stated that an increase in N fertilisation will result in a lower clover component. Furthermore, Harris and Clark (1996) determined that the clover component of a grass-clover pasture should always be kept above a threshold value of 30%. This will ensure that the clover component adequately contributes to N fixation and pasture quality. Throughout the trial period, however, any N application, even at relatively low rates, led to the clover component decreasing below this threshold value. The ryegrass, as well as the kikuyu component, was positively related to N fertilisation rates in their respective active growing seasons *viz*. winter and spring for ryegrass and summer and autumn for kikuyu. This is in agreement with literature that states that ryegrass and kikuyu growth yield increases with N fertilisation rate (Whitney 1974; Wilkins 1991; Ledgard *et al.* 2001). The negative relationship between the clover component and N fertilisation rate can be explained by the increase in grass growth (Ledgard *et al.* 2001). According to Ledgard and Steele (1992), grasses generally grow taller and faster under high N fertilisation rates than legumes. Consequently, the grass out competes the legume for sunlight, nutrients and water. The grasses therefore had a competitive advantage over the clover and supressed clover growth as N fertilisation rates increased (Ledgard and Steele 1992; Shiel *et al.* 1999; Ledgard *et al.* 2001; Nyfeler *et al.* 2011).

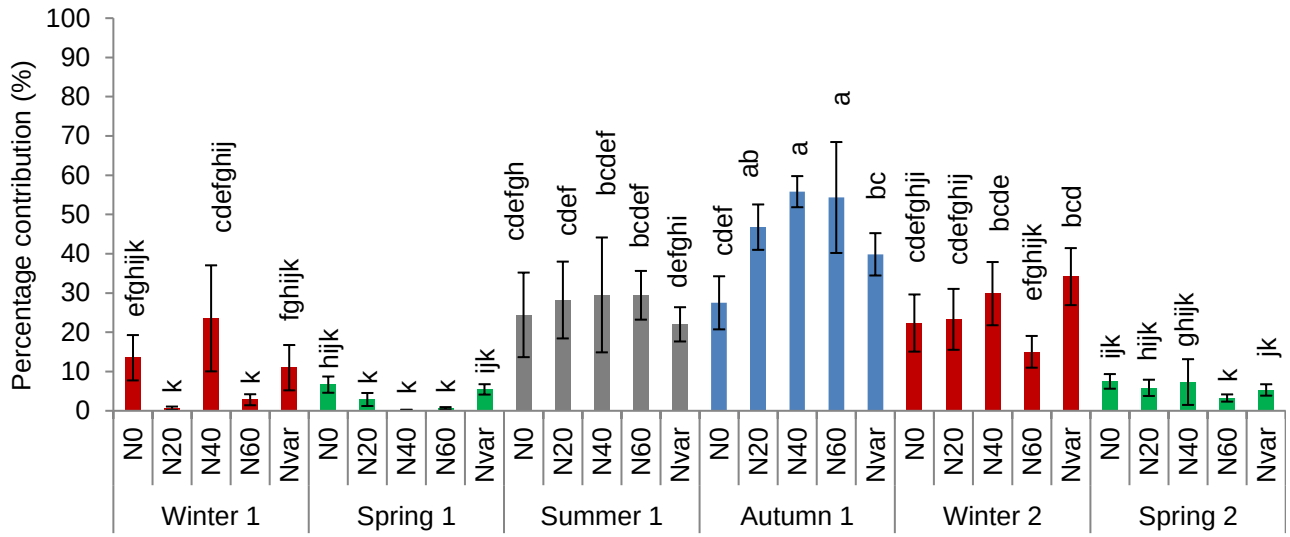

Figure 4.3.2. Seasonal changes in kikuyu's contribution to botanical composition in response to nitrogen fertilisation treatments that were applied after grazing. N0, N20, N40, N60 = 0, 20, 40, 60 kg N ha^{-1}; N_{var} = variable nitrogen fertilisation. Error bars indicate standard error. No common letter above bars denotes a significant difference (P<0.05)

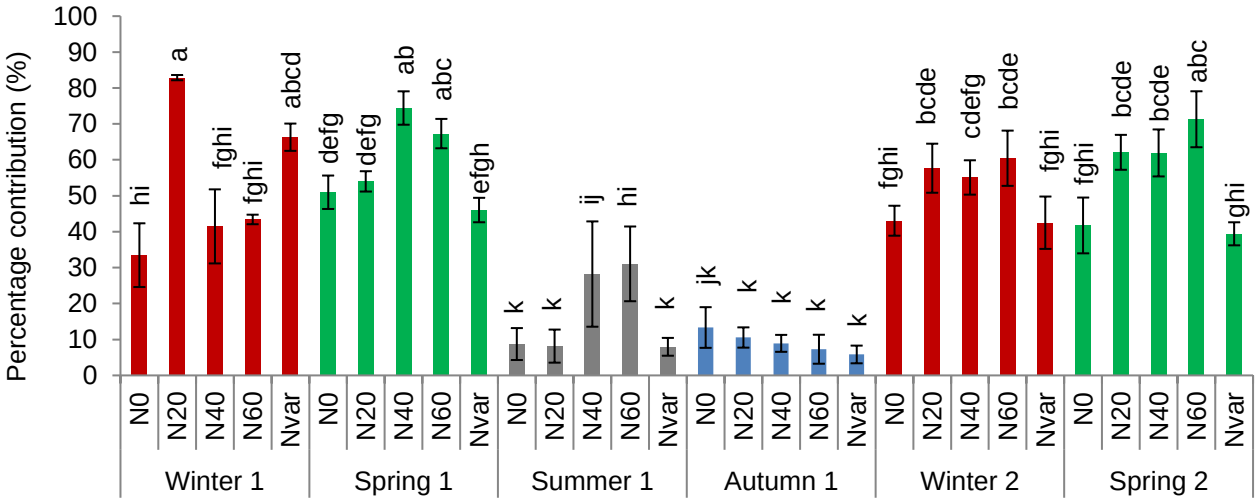

Figure 4.3.3. Seasonal changes in ryegrass's contribution to botanical composition in response to nitrogen fertilisation treatments that were applied after grazing. N0, N20, N40, N60 = 0, 20, 40, 60 kg N ha^{-1}; N_{var} = variable nitrogen fertilisation. Error bars indicate standard error. No common letter above bars denotes a significant difference (P<0.05).

Figure 4.3.5 and Figure 4.3.6 represent pasture species that were not over-sown into the sward. Figure 4.3.5 shows the weed component whilst Figure 4.3.6 shows the component comprising other grasses. Weeds were only affected by N fertilisation treatment in the winter of year one, were N40 and N60 had an equal ($P > 0.05$) weed components but were higher ($P \leq 0.05$) than the rest. From spring in year one to spring in year two, the weed component was constant ($P > 0.05$) and was not influenced by N fertilisation. The other grass component for all treatments, except N60, increased ($P \leq 0.05$) from winter to summer (Figure 4.3.6). In the summer treatment N20 had a

higher ($P \leq 0.05$) other grass component than N40 and N60. From summer in year one to the spring in year two the other grass components for all treatments decreased ($P \leq 0.05$) except N40 and N60. High fertilisation rates may thus favour the incursion of pastures by poor quality, weedy grasses such as *Paspalum*, *Eragrostis plana* and *Sporobolus africana* during summer and autumn.

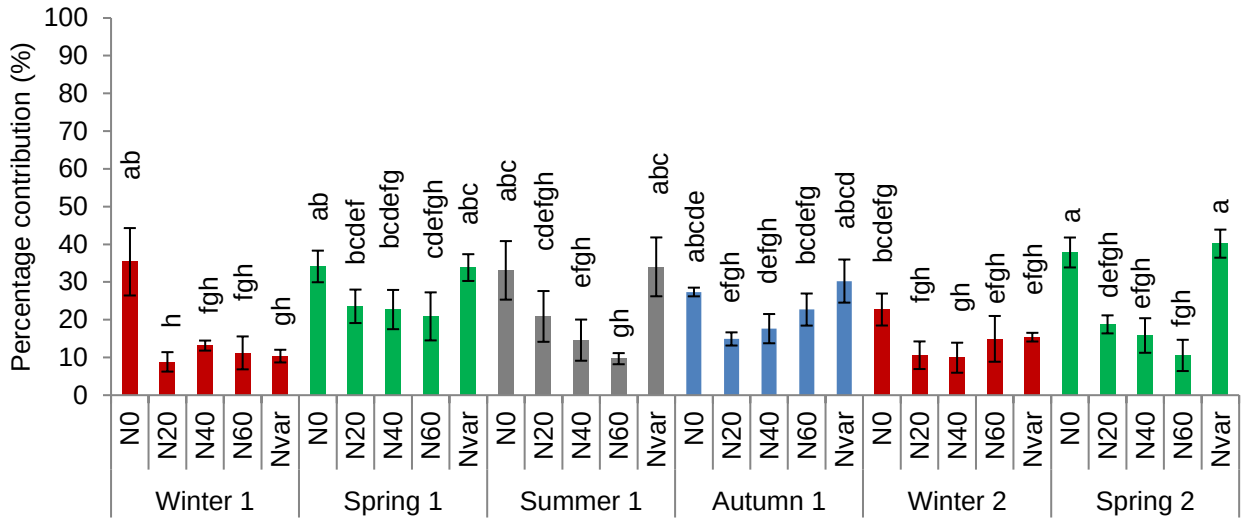

Figure 4.3.4. Seasonal changes in clover's contribution to botanical composition in response to nitrogen fertilisation treatments that were applied after grazing. N0, N20, N40, N60 = 0, 20, 40, 60 kg N ha^{-1}; N_{var} = variable nitrogen fertilisation. Error bars indicate standard error. No common letter above bars denotes a significant difference (P<0.05).

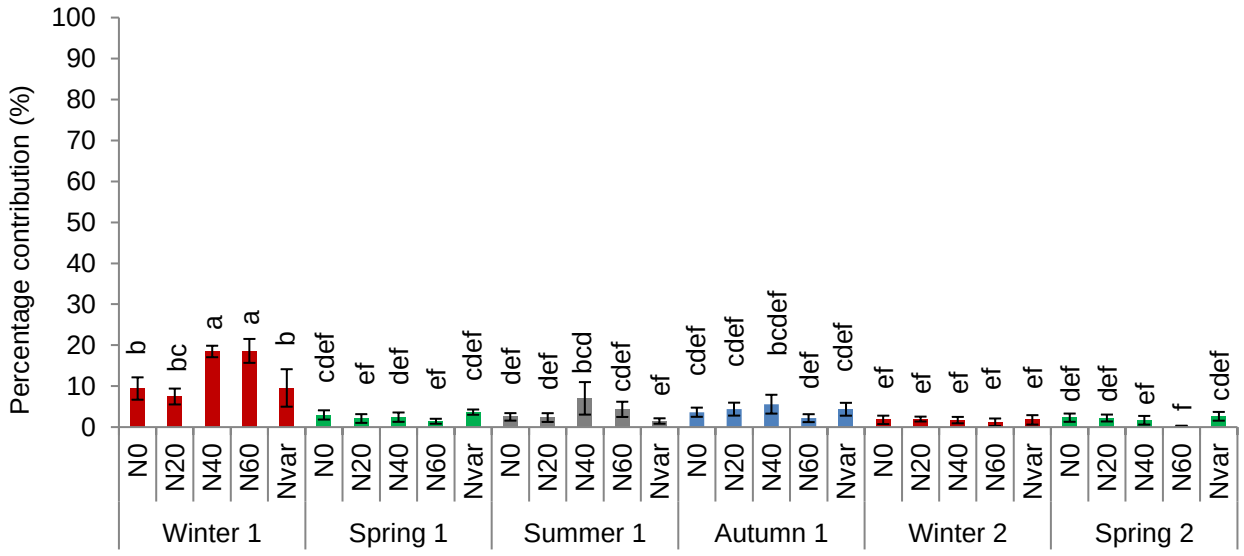

Figure 4.3.5. Seasonal changes in weeds' contribution to botanical composition in response to nitrogen fertilisation treatments that were applied after grazing. N0, N20, N40, N60 = 0, 20, 40, 60 kg N ha^{-1}; N_{var} = variable nitrogen fertilisation. Error bars indicate standard error. No common letter above bars denotes a significant difference (P<0.05).

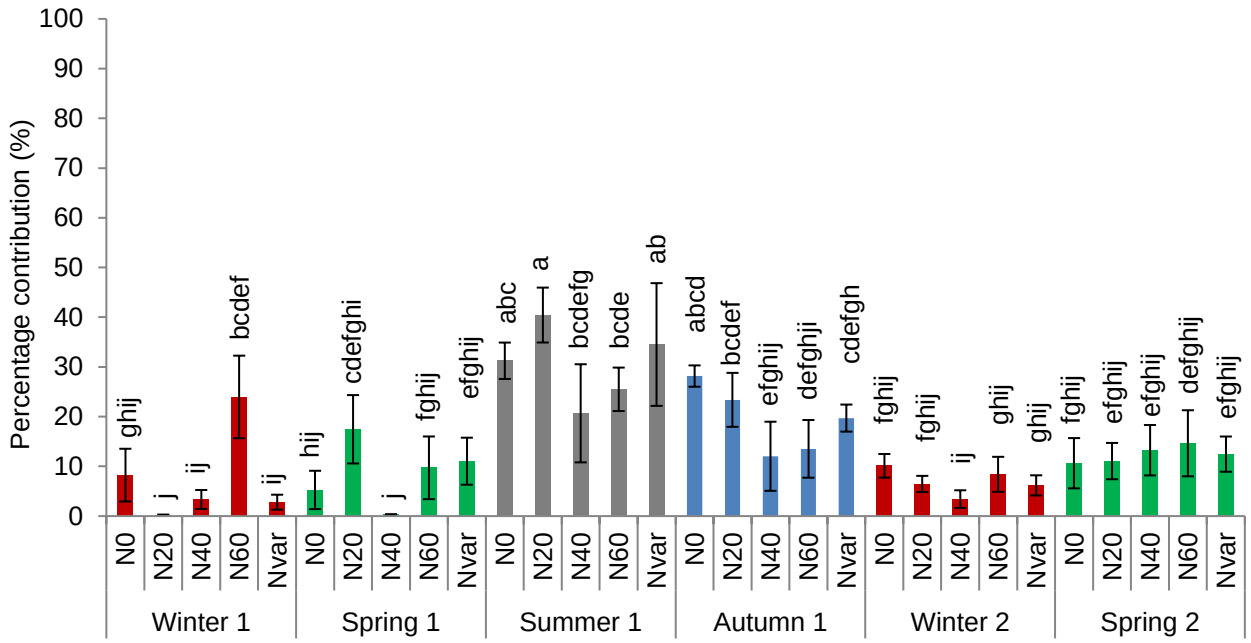

Figure 4.3.6. Seasonal changes in other grass's contribution to botanical composition in response to nitrogen fertilisation treatments that were applied after grazing. N0, N20, N40, N60 = 0, 20, 40, 60 kg N ha^{-1}; N_{var} = variable nitrogen fertilisation. Error bars indicate standard error. No common letter above bars denotes a significant difference (P<0.05).

From the spring in year one to the winter in year two the weed component was low in comparison to other pasture components. Therefore, weeds would not have significantly influenced pasture quality or milk production. However, other grasses ranged between 12 and 40% from summer to autumn and could have contributed to pasture quality, yield and milk production in these seasons.

The effect of N fertilisation through time on botanical composition is illustrated as a non–metric multidimensional scaling ordination in Figure 4.3.7. For the interpretation of the ordination plots, the distance between the data points are of interest rather than the position of each point. For example, the points that are the closest in proximity are related, whilst those furthest apart are unrelated. The different shapes show the different seasons, while the different colours represent the N fertilisation treatments. The arrows display linear correlations between variables. The effect of N fertilisation rate is shown by the blue arrow. The green arrows show the season that the pasture species were highly associated with. The length of the arrow indicates the strength of the correlation. The seasonal effect was stronger than the effect of N fertilisation. The influence of N fertilisation rate only accounted for 6% of the variation (R^2= 0.06). The arrow indicating the effect of N fertilisation, shows that the clover component was slightly positively correlated to N fertilisation rates in the spring, whilst the kikuyu component was slightly negatively related to N fertilisation rates in the autumn. This could be explained by the total amount of N fertiliser that was applied per season. In the spring more N was applied than in the winter and autumn and clover growth was also high in spring (Table 4.2.3). This may have resulted in the positive correlation between clover

and N fertilisation in the spring. Although N fertilisation did influence botanical composition, seasonal effects were the main determining factor for changes in botanical composition.

The arrow indicating the clover and ryegrass components points in the direction of the spring. The arrow indicating the kikuyu component points in the direction of the autumn. The clover and ryegrass components were therefore prominent in the spring and the kikuyu component in the autumn. Clover and ryegrass actively grow in the spring, whilst kikuyu actively grows in the summer and autumn. Therefore these results were as expected within a kikuyu-based system (Botha *et al.* 2008a; Chapman *et al.* 2011; Van der Colf *et al.* 2015b). Furthermore, the weed and other grass components were prominent in the winter and summer, respectively.

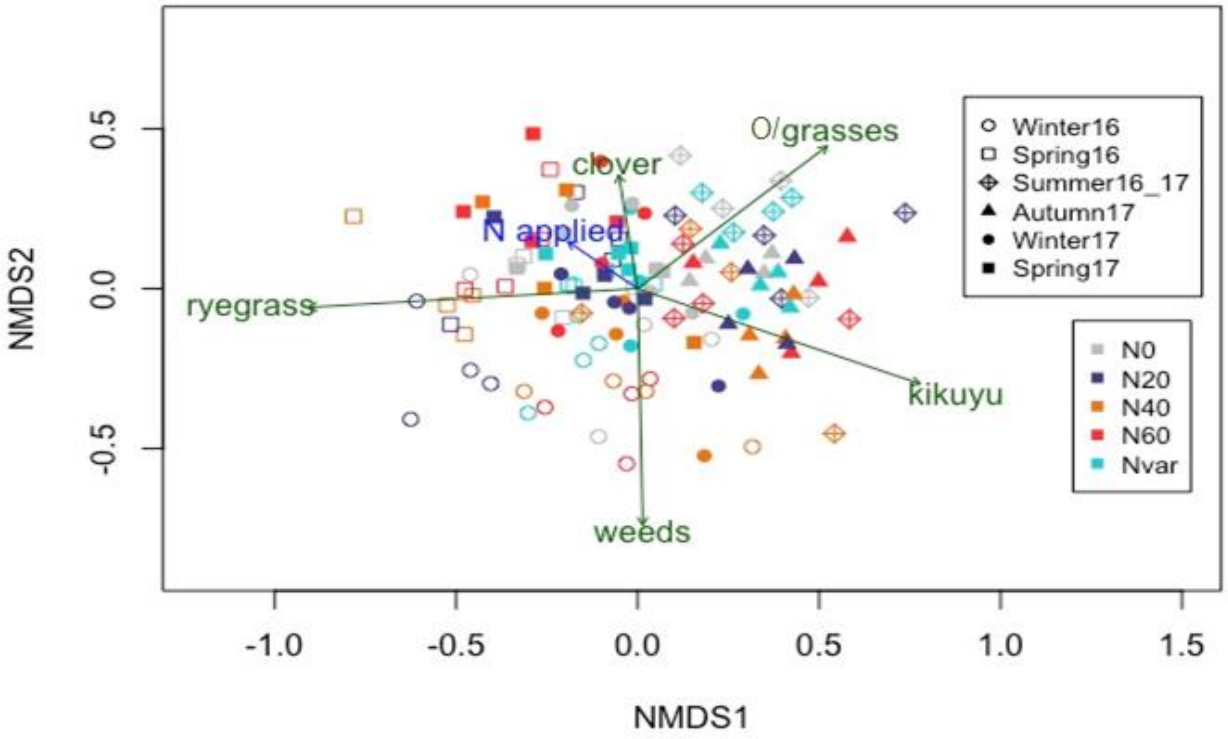

Figure 4.3.7. Non–metric multidimensional scaling ordination of the effect of N fertilisation treatments, applied after grazing, on pasture species composition over seasons during year one. N0, N20, N40, N60 = 0, 20, 40, 60 kg N ha^{-1}; N_{var} = variable N application. Axis 1 and 2. R^2 = 0.06, P = 0.023.

4.3.3 Kikuyu-perennial ryegrass-clover crude protein

Figure 4.3.8 shows the effect of N fertilisation regimes on herbage crude protein (CP) content (%) of KPrCl. Treatment N60 had the highest or similar to the highest ($P \leq 0.05$) CP content during all months. In October, November, February and March the CP content of N60 was higher ($P \leq 0.05$) than for N0. In November, February, January and March the CP content of N60 was higher ($P \leq 0.05$) than for N_{var}. This is in accordance with literature that states that N content of herbage

increases as N fertilisation rate increases (Smith and Sund 1965; Shiel *et al.* 1999). Botha *et al.* (2008) measured the CP content of a kikuyu-perennial ryegrass-clover pasture that received no N. This study reported a crude protein content of c. 23% in the winter and spring and c. 18.5 % in the summer and autumn. The same trend was found under treatment N0 and N_{var}. Both N0 and N_{var} produced a lower crude protein content in summer (c.18%) and higher in winter (c. 21%).

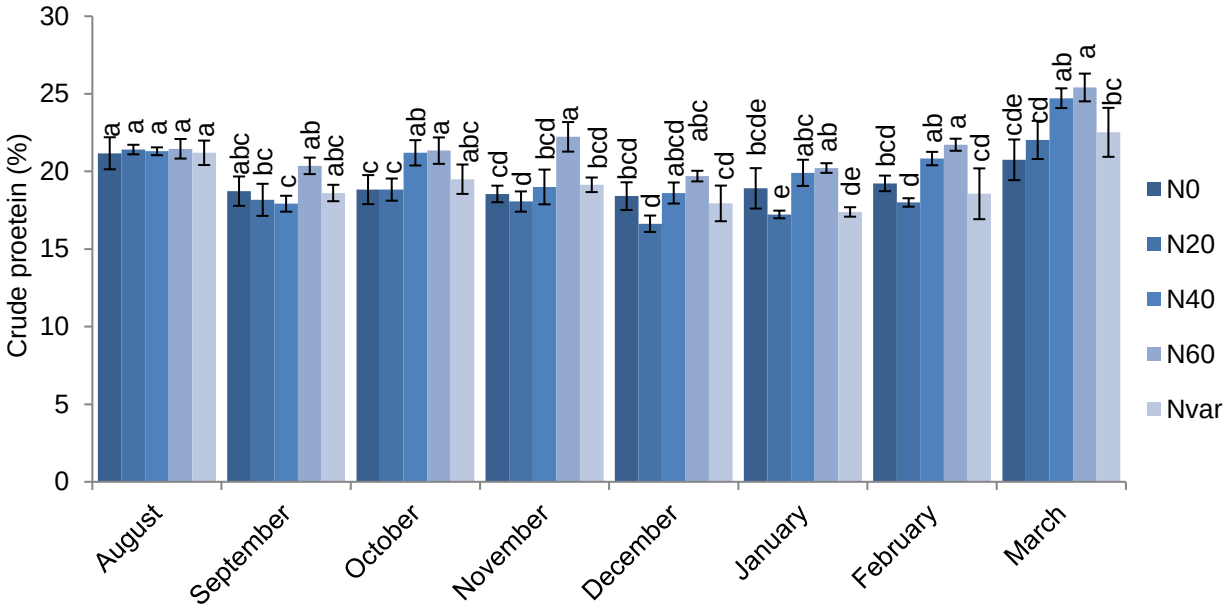

Figure 4.3.8. Monthly changes in herbage crude protein content in response to nitrogen fertilisation treatments that were applied after grazing. N0, N20, N40, N60 = 0, 20, 40, 60 kg N ha^{-1}; N_{var} = variable nitrogen fertilisation. Treatments were compared within months. Error bars indicate standard error. No common letter above bars denotes a significant difference within months (P<0.05).

4.3.4 Kikuyu-lucerne herbage production

In September of year one treatment had no effect ($P > 0.05$) on yield (Table 4.3.3). In October and November treatment N60 and N20 produced a higher yield ($P \leq 0.05$) than N_{var} respectively. In January treatment had no effect ($P > 0.05$) on yield. Treatment N60 out yielded ($P \leq 0.05$) N0 in all months from February to July, except in May. In May N40 was higher ($P \leq 0.05$) than N0. In September of year two, treatment N40 again out-yielded ($P \leq 0.05$) N0. In October there were no differences ($P > 0.05$) in herbage yield between fertilisation treatments. In November the yield of N60 was equal to N40 but higher ($P \leq 0.05$) than the rest. Treatment N60 was the only treatment that maintained the highest ($P \leq 0.05$) or similar ($P > 0.05$) to the highest yield throughout all months during the study.

Table 4.3.4 shows the seasonal yield and illustrates that for all treatments, herbage yield increased ($P \leq 0.05$) from winter to spring as well as from spring to summer of year one. From summer to autumn, of year one, herbage yield decreased ($P \leq 0.05$) for all treatments. From autumn in year one until winter of year two the yield for all treatments, except N20 and N40, remained constant ($P > 0.05$). From winter to spring in year two, the yield under all treatments increased ($P \leq 0.05$). In year one, herbage yield in winter did not differ ($P > 0.05$) between treatments. In spring and

summer, N60 had highest or similar to the highest ($P \leq 0.05$) herbage yield. In autumn the yield of N0 and N_{var} were lower ($P \leq 0.05$) than the rest. In year two, treatment had no effect on yield in the winter. The herbage yield under N60 was higher ($P \leq 0.05$) than for N_{var} in the spring. The effect of N fertilisation on monthly as well as seasonal yield reflects in the annual yield (Figure 4.3.9). Treatment N20, N40 and N60 had higher ($P \leq 0.05$) total annual herbage yields than N0 and N_{var}, therefore indicating that N fertilisation rate had an effect on herbage yield. There were no differences between the N20, N40 and N60 fertilisation regimes.

Brown and Moot (2004) reported that a pure lucerne pasture, that received no N, produced an annual average yield of 21 t DM ha^{-1} yr^{-1}. For a kikuyu pasture Swanepoel *et al.* (2014) reported a yield of 21.3 t DM ha^{-1} yr^{-1} at N fertilisation regime of c. 360 kg N ha^{-1} yr^{-1}. These yields were similar to the average annual yield of treatment N20, N40 and N60 found in this study (20.9 t DM ha^{-1} yr^{-1}). Fourie (2015) reported that a kikuyu-lucerne pasture fertilised with no N, yielded c. 19 t DM ha^{-1} yr^{-1}. The annual yields for treatment N0 and N_{var} (19.2 and 18.9 t DM ha^{-1} yr^{-1}) were similar to yields found by Fourie (2015).

Similarly to the KPrCl site (4.3.1), animal excreta could have influenced yield. As a result of grazing practises, N from excreta was distributed more or less equally throughout the experimental site. The N from excreta may have influenced the effect of the N treatments.

Table 4.3.3. The total monthly yield (kg DM ha^{-1} yr^{-1}) of a kikuyu-lucerne pasture in response to nitrogen fertilisation treatments applied after grazing. N0, N20, N40, N60 = 0, 20, 40, 60 kg N ha-1; Nvar =variable nitrogen fertilisation. Treatments were compared within months. No common letter denotes a significant difference (P<0.05).

		Nitrogen treatment				
Year	**Month**	**N0**	**N20**	**N40**	**N60**	**N_{var}**
1	September	2386^{c}	2501^{abc}	2428^{bc}	2401^{c}	2339^{c}
	October	2633^{cd}	2669^{bcd}	2832^{bcd}	3033^{ab}	2604^{d}
	November	3004^{ab}	3338^{ab}	2487^{d}	2912^{bc}	2595^{cd}
	January	2659^{abc}	2508^{bc}	2502^{bc}	2903^{abc}	2354^{c}
	February	2117^{c}	2252^{bc}	2484^{ab}	2504^{ab}	2421^{abc}
	March	2045^{d}	2132^{cd}	2377^{bcd}	2583^{ab}	2049^{d}
	May	1480^{d}	1825^{bcd}	2070^{ab}	1775^{bcd}	1585^{d}
	June	1213^{cd}	1666^{b}	1735^{ab}	1612^{b}	1186^{d}
2	July	832^{c}	1222^{ab}	1040^{bc}	1210^{ab}	829^{c}
	September	1442^{bcd}	1552^{abc}	1816^{a}	1643^{ab}	1500^{abcd}
	October	2088^{bc}	2135^{abc}	2312^{ab}	2140^{abc}	2113^{bc}
	November	1953^{bcd}	1868^{cd}	2209^{abc}	2352^{a}	1853^{cd}

^{abcd}Means without a common superscript differed significantly (P<0.05).

Table 4.3.4. The total seasonal herbage yield (kg DM ha^{-1}) of a kikuyu-lucerne pasture in response to nitrogen fertilisation treatments applied after each grazing. N0, N20, N40, N60 = 0, 20, 40, 60 kg N ha^{-1}; N_{var}=variable nitrogen fertilisation.

	Nitrogen fertilisation treatment				
Season	**N0**	**N20**	**N40**	**N60**	**N_{var}**
Winter 1	4067^{efg}	4182^{efg}	4109^{efg}	4081^{efg}	4020^{efgh}
Spring 1	5637^{cd}	6008^{c}	5321^{d}	5945^{c}	5200^{d}
Summer 1	6822^{b}	6893^{b}	7365^{b}	7992^{a}	6826^{b}
Autumn 1	2695^{kl}	3492^{hi}	3806^{ghi}	3388^{ij}	2772^{kl}
Winter 2	2275^{kl}	2775^{kl}	2856^{jk}	2854^{jk}	2330^{kl}
Spring 2	4042^{efg}	4003^{efgh}	4520^{e}	4492^{e}	3966^{fgh}

^{abcd}Means without a common superscript differed significantly (P<0.05).

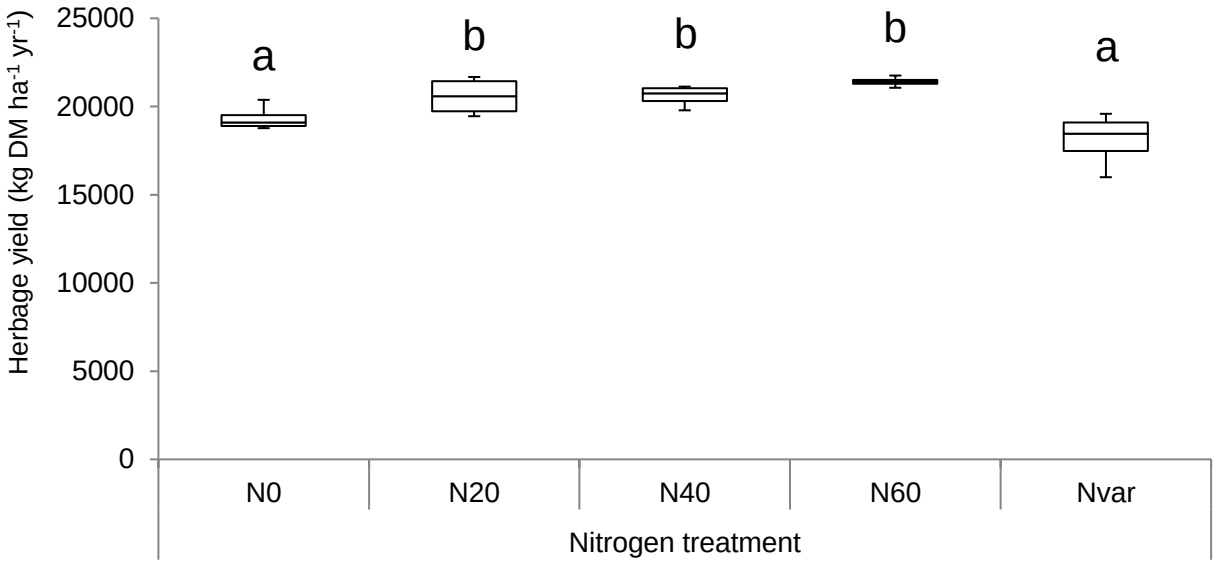

Figure 4.3.9. The total annual herbage yield (kg DM ha^{-1} yr^{-1}) of a kikuyu-lucerne pasture, during year one, in response to nitrogen fertilisation treatments applied after grazing. N0, N20, N40, N60 = 0, 20, 40, 60 kg N ha^{-1}; N_{var} = variable nitrogen fertilisation. Error bars indicate standard error. No common letter above bars denotes a significant difference (P<0.05).

4.3.5 Kikuyu-lucerne botanical composition

The effect of N fertilisation on kikuyu and lucerne's contribution to botanical contribution is shown in Figure 4.3.10 and Figure 4.3.11 respectively. During year one and two, treatment had no effect ($P > 0.05$) on the kikuyu as well as the lucerne component within season, except in the summer were the lucerne component of N40 was higher ($P \leq 0.05$) than the rest. The kikuyu and lucerne component, under all treatments, increased ($P \leq 0.05$) from winter in year one to autumn in year one and decreased ($P \leq 0.05$) from autumn in year one to winter in year two.

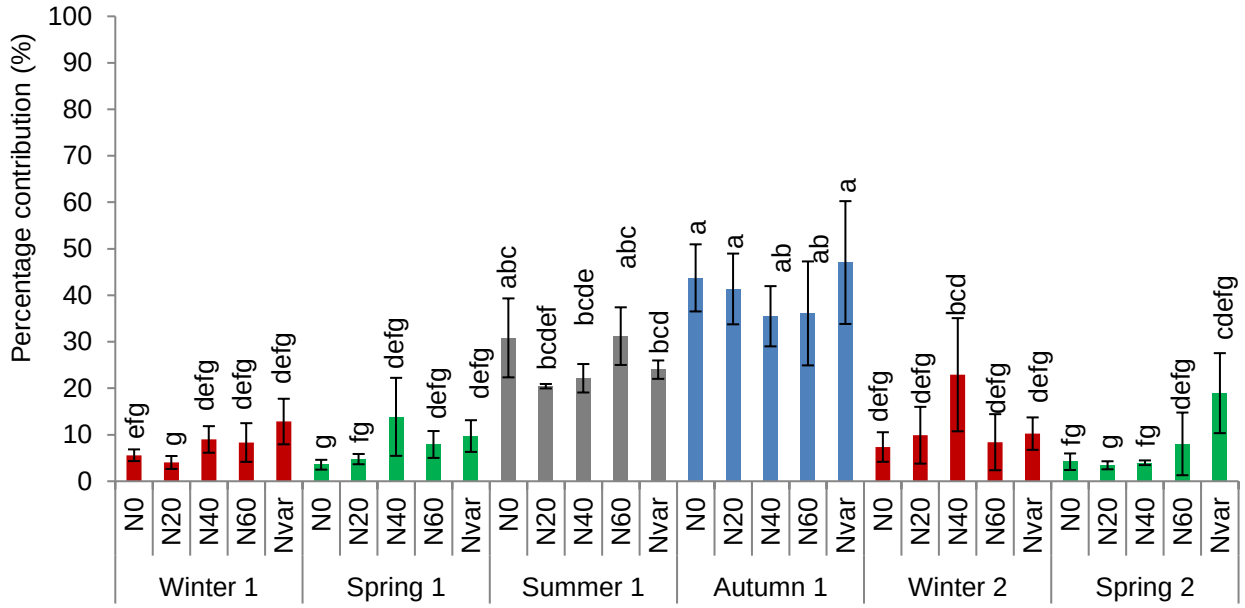

Figure 4.3.10. Seasonal changes in kikuyu's contribution to botanical contribution in response to nitrogen fertilisation treatments that were applied after grazing. N0, N20, N40, N60 = 0, 20, 40, 60 kg N ha^{-1}; N_{var} = variable nitrogen fertilisation. Error bars indicate standard error. No common letter above bars denotes a significant difference (P<0.05).

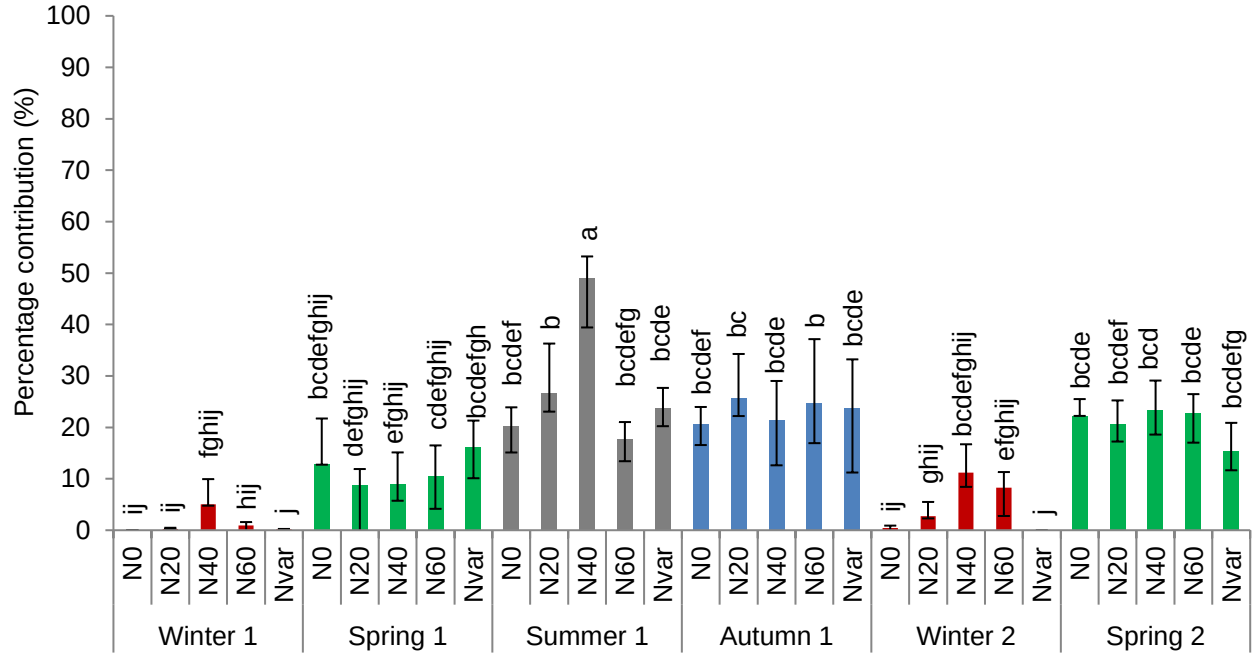

Figure 4.3.11. Seasonal changes in lucerne's contribution to botanical contribution in response to nitrogen fertilisation treatments that were applied after grazing. N0, N20, N40, N60 = 0, 20, 40, 60 kg N ha^{-1}; N_{var} = variable nitrogen fertilisation. Error bars indicate standard error. No common letter above bars denotes a significant difference (P<0.05).

The effect of N fertilisation on other grass is illustrated in (Figure 4.3.12). Treatment had no effect ($P > 0.05$) on the other grass component except in winter of year two, where the other grass component for N20 and N60 was higher ($P \leq 0.05$) than N40. For year one, the other grass component of all treatments decreased ($P \leq 0.05$) from spring to autumn. From autumn in year one to spring in year two, the other grass component for all treatments except N_{var} increased ($P \leq 0.05$).

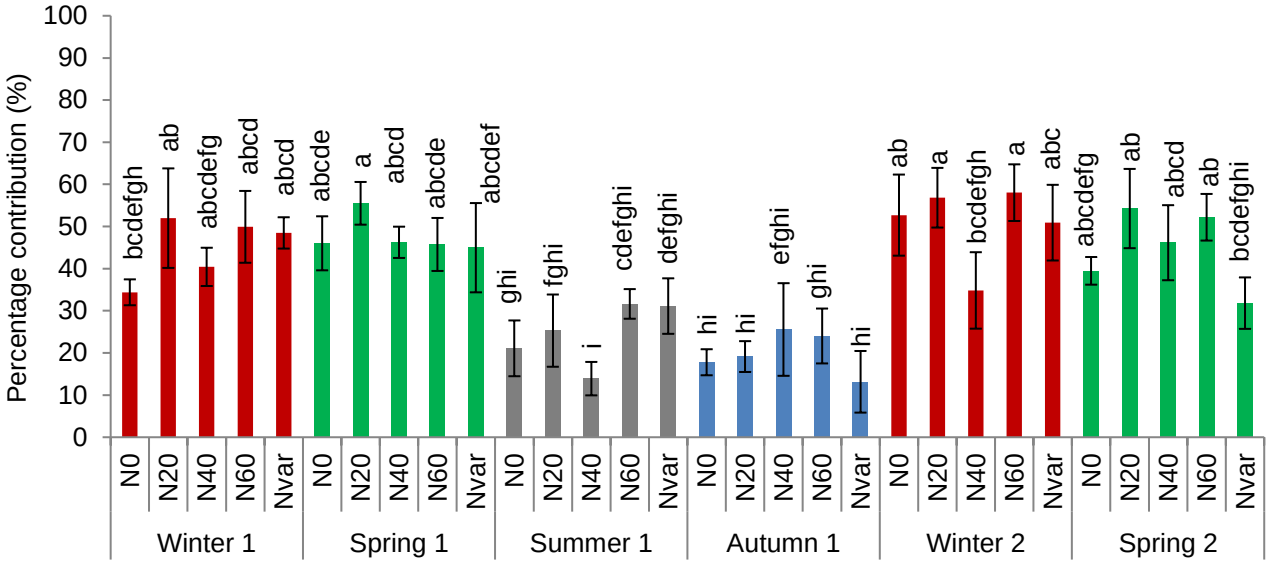

Figure 4.3.12. Seasonal changes in other grass's contribution to botanical contribution in response to nitrogen fertilisation treatments that were applied after grazing. N0, N20, N40, N60 = 0, 20, 40, 60 kg N ha^{-1}; N_{var} =variable nitrogen fertilisation. Error bars indicate standard error. No common letter above bars denotes a significant difference (P<0.05).

The other legume component in the kikuyu-lucerne pastures consisted predominately of white clover (*Trifolium repens*). In the winter of year one, the other legume component for treatment N60 was lower ($P \leq 0.05$) than that of N0 (Figure 4.3.13). From winter to spring the other legume component for all treatments remained constant ($P > 0.05$), except N60 where it increased ($P \leq 0.05$). From spring to summer the other legume component for N40 and N60 decreased, while the rest remained constant. From summer in year one to spring in year two, the other legume component for all treatments did not differ ($P > 0.05$).

The weed component in the winter of year one was higher ($P \leq 0.05$) under treatment N0 than N20 and N_{var}. From winter year one to spring year one the weed component for all treatments decreased ($P \leq 0.05$). From spring to autumn, treatment had no effect ($P > 0.05$) on the weed component. From autumn in year one to winter in year two, the weed component for all treatments increased ($P \leq 0.05$). From winter year two, to spring year two the weed components for N0 and N20 decreased ($P \leq 0.05$) while the rest stayed constant ($P > 0.05$).

Although this pasture type is referred to as a kikuyu-lucerne pasture, the kikuyu and lucerne components only dominated the sward during the summer and autumn. In the winter and spring,

the sward mainly consisted of other grasses, other legumes and weeds. This may be explained by the growth pattern of both kikuyu and lucerne. Kikuyu is dormant in the winter and actively grows from summer to autumn, whilst the lucerne cultivar that was used was a winter dormant type (Marais 2001). The dormancy of kikuyu and lucerne could have resulted in other grasses, other legumes and weeds dominating the pasture during winter and spring. Although no other pasture species were established with commencement of this study, the trial area was previously utilised as a kikuyu-ryegrass or kikuyu-ryegrass-clover pasture. The other grasses component was therefore mostly ryegrass and other legumes were predominantly clover.

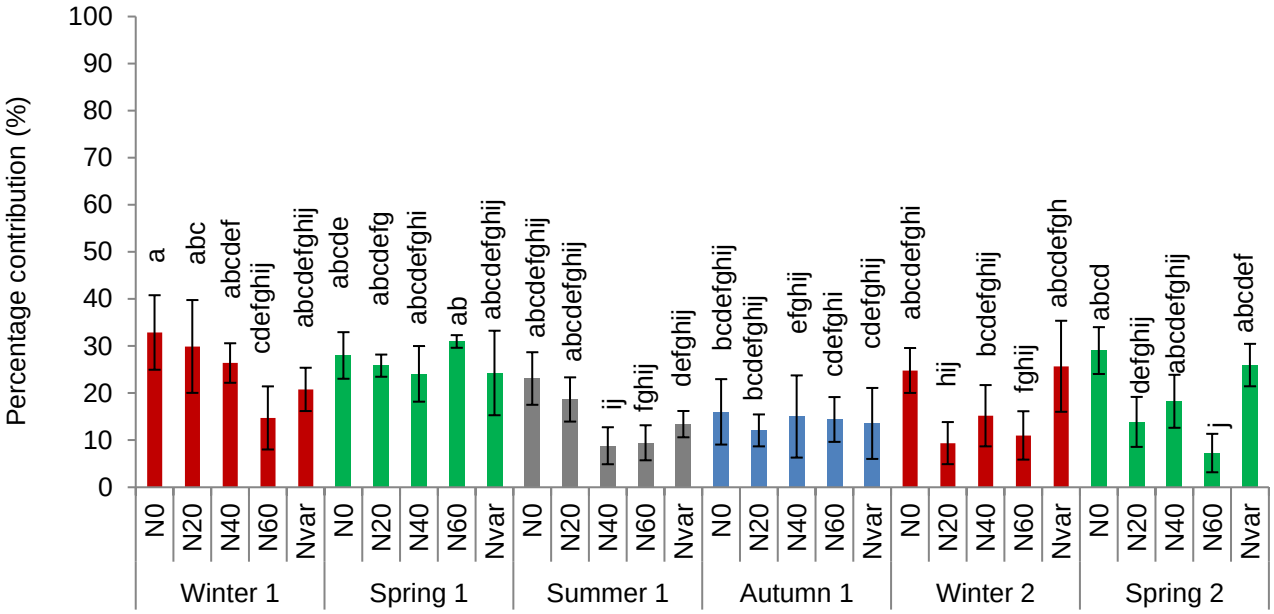

Figure 4.3.13. Seasonal changes in other legumes' contribution to botanical contribution in response to nitrogen fertilisation treatments that were applied after grazing. N0, N20, N40, N60 = 0, 20, 40, 60 kg N ha^{-1}; N_{var} =variable nitrogen fertilisation. Error bars indicate standard error. No common letter above bars denotes a significant difference (P<0.05).

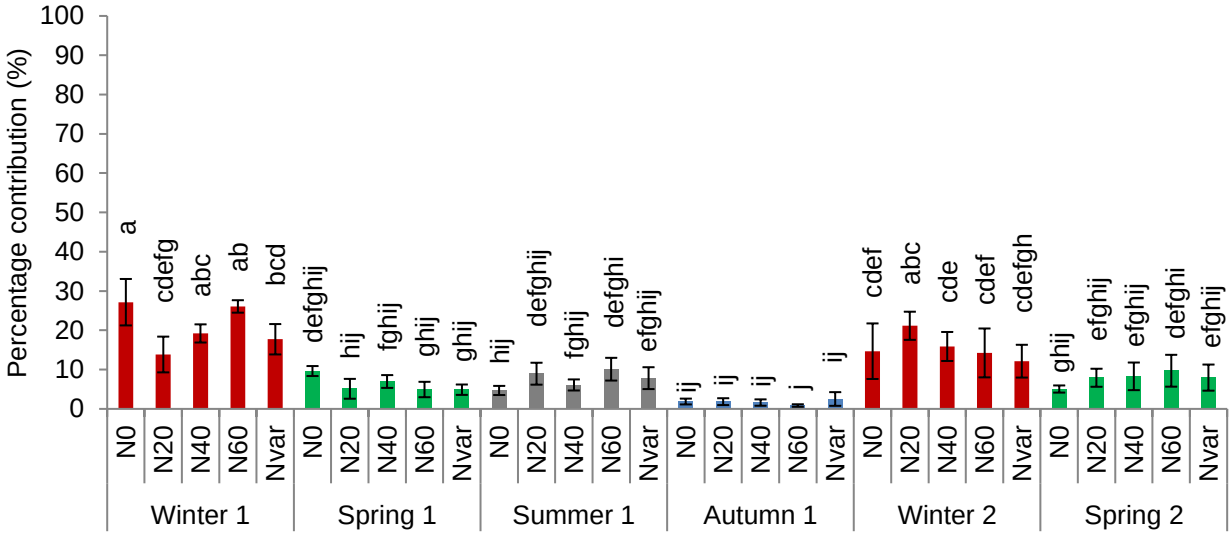

Figure 4.3.14. Seasonal changes in weeds' contribution to botanical contribution in response to nitrogen fertilisation treatments that were applied after grazing. N0, N20, N40, N60 = 0, 20, 40, 60 kg N ha^{-1}; N_{var} =variable nitrogen fertilisation. Error bars indicate standard error. No common letter above bars denotes a significant difference (P<0.05).

Unlike the kikuyu component of the KPrCl pasture site, the kikuyu component for the KL site was not influenced by N fertilisation treatment. Since lucerne and kikuyu actively grow during the same period, competition for resources occurs (Marais 2001). Unlike white clover, lucerne grows upright and possesses a taproot system that can penetrate the soil up to 4m deep (Langer 1990; Charlton and Stewart 1999; Brown *et al.* 2003). The lucerne component may have thus out-competed the kikuyu for water, nutrients and sunlight and therefore limited kikuyu's growth.

Fourie (2015) studied a kikuyu-lucerne pasture, under grazing, in close proximity to this study. No N was applied to the pasture and therefore the results are comparable to treatment N0 and N_{var}. Fourie (2015) reported a lucerne component c. 10% higher, for all seasons, than that of N0 and N_{var}. Fourie (2015) also found a smaller other grass, other legumes and weeds (invasive pasture species) component. The lucerne component reported in this study could therefore have been negatively affected by the high percentage of invasive species. The establishment of lucerne in autumn, followed by competition from invasive pasture species in winter might have resulted in poor emergence, therefore reducing the lucerne component.

The non–metric multidimensional scaling ordination on the effect of N fertilisation on pasture species composition is illustrated in Figure 4.3.15. Similarly to the KPrCl pasture type, the effect of seasonal change on botanical composition was more significant than the effect of N fertilisation rate. The blue arrow indicates the effect of N fertilisation on botanical composition. The other legume component was therefore negatively correlated to N fertilisation rates in the spring, whilst the lucerne component was positively correlated to N fertilisation rates in summer.

The green arrows illustrate the different pasture species components. The arrows point in the direction of the season that the pasture species highly associates with. The arrows of the kikuyu and lucerne components point in the direction of autumn and summer respectively. Kikuyu and lucerne actively grow in the autumn and summer respectively and therefore these findings were anticipated (Figure 4.3.15) (Marais 2001; McDonald *et al*. 2003; Van der Colf *et al.* 2015b). Both the other grass and other legume components were associated with spring and winter. Since other grasses were mainly ryegrass and other legumes were mainly clover, these findings agree with the growing periods in which ryegrass and clover would actively grow.

4.3.6 Kikuyu-lucerne crude protein

The effect of N fertilisation treatments on crude protein is shown in Figure 4.3.16. From September to January treatment had no effect on crude protein. From February to June the KL pasture type followed the same trend as the KPrCl pasture type, where crude protein for treatment N60 was highest or similar to the highest ($P \leq 0.05$). The lowest crude protein found for KL pasture type was

17 % and the highest was 27 %. This was comparable to the 16 and 26% found for the KPrCl pasture type respectively. Crude protein was similar for both pasture types since the legume component of the KL pasture type, lucerne, generally has similar crude protein content to the legume of the KPrCl type, clover (McDonald *et al*. 2003). Additionally, the KL pasture type had a significant other legume component, mainly clover, throughout the study.

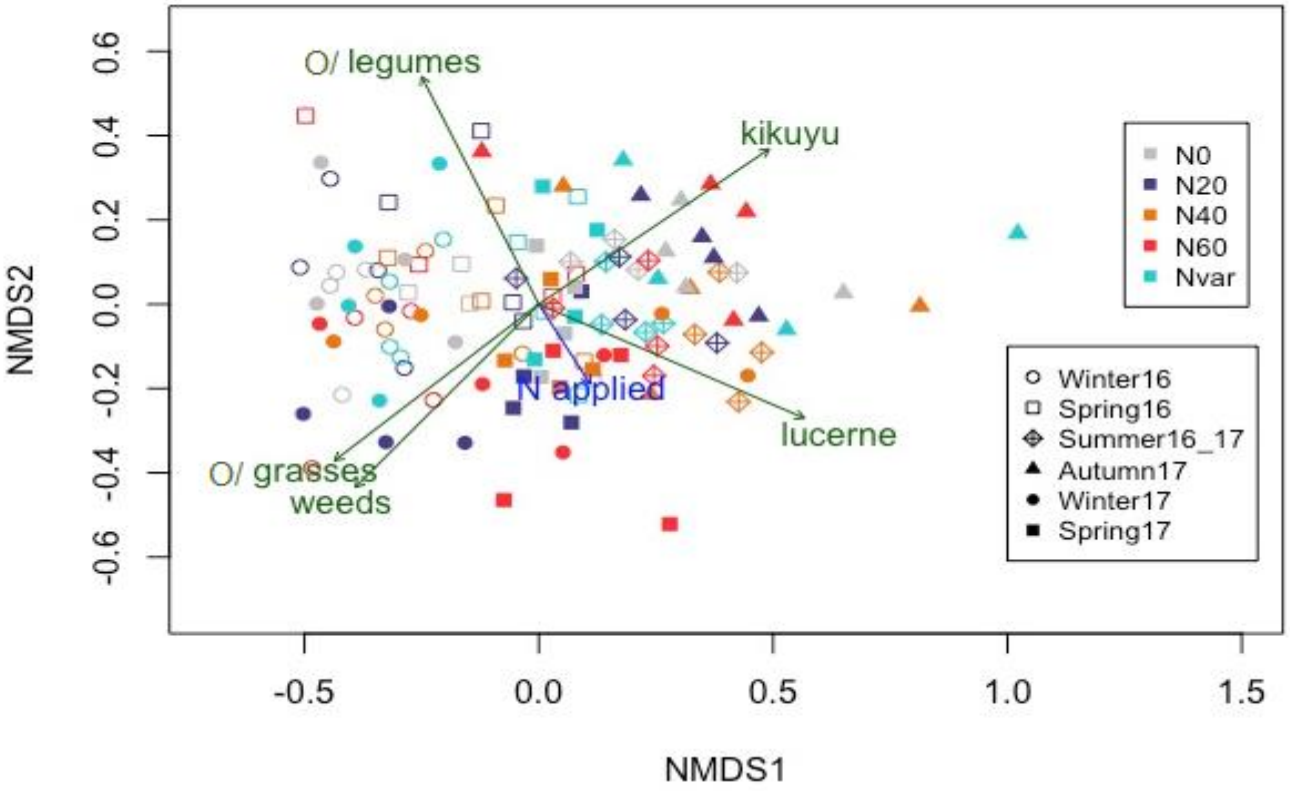

Figure 4.3.15. Non–metric multidimensional scaling ordination of the effect of N fertilisation treatments (applied after grazing) on pasture species composition over seasons, during year one. N0, N20, N40, N60 = 0, 20, 40, 60 kg N ha^{-1}; N_{var} = variable N application. Axis 1 and 2. R^2 0.07, P = 0.015.

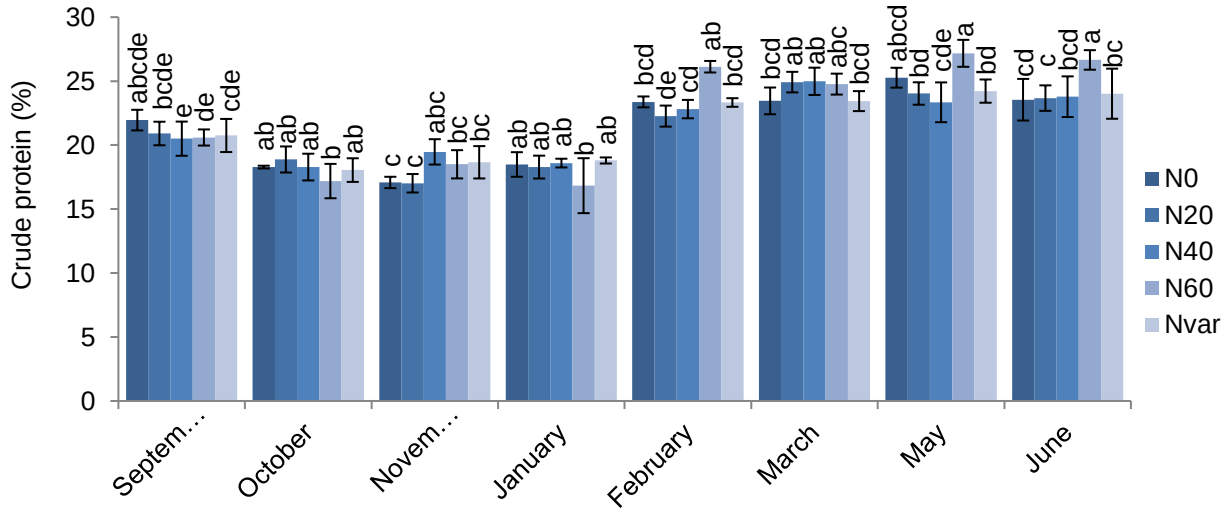

Figure 4.3.16. Monthly changes in herbage crude protein content in response to nitrogen fertilisation treatments that were applied after grazing. N0, N20, N40, N60 = 0, 20, 40, 60 kg N ha^{-1}; N_{var} = variable nitrogen fertilisation. Treatments were compared within months. Error bars indicate standard error. No common letter above bars denotes a significant difference within months ($P<0.05$).

4.4 Conclusion and recommendations

For the KPrCl pasture type, annual yield was not affected by N fertilisation rate. Treatment only affected yield in the spring of year one where N60 produced a higher yield than N0 and N_{var}, but was equal to other treatments. This indicates that N0 was sufficient for optimal herbage production in the summer, winter and autumn. However, in spring it may be beneficial to apply N. With regards to botanical composition, the kikuyu and ryegrass components were positively related to N fertilisation rates in autumn and winter respectively. The clover component tended to be promoted by the N0 and N_{var} regimes throughout the study. These results show that N0, or nil N application regimes, are preferred for the promotion of a high clover content, while high N rates will result in a higher grass component in kikuyu-ryegrass-clover pastures. Crude protein content for the KPrCl pasture was the highest or similar to the highest under treatment N60 in all months. This shows that N accumulates in the plant as N fertilisation rates increased under treatment N60, and that N supply might surpass N demand. Over-fertilisation may be occurring under treatment N60.

For the KL pasture, annual yield for treatment N20, N40 and N60 was higher than N0 and N_{var}. Treatment N60 produced the highest or similar to the highest seasonal yield in the spring, summer and autumn. This indicates that a higher N fertilisation regime (N40 and N60) is preferred for the seasons of spring, summer and autumn, while lower N rates (N0 and N20) may be sufficient for herbage production in the winter. With regards to botanical composition, N had no effect on the kikuyu component in the KL pasture. The lucerne component was not affected by N fertilisation, except in the summer were N40 produced the highest lucerne component. It may be beneficial to apply N40 in the summer to promote growth of the lucerne component. The crude protein content of KL was not affected by N fertilisation rates from September to January. From February to June the N60 treatment resulted in a crude protein content that was higher than the other treatments or similar to the highest. This indicates that in these months N accumulated in the plant and that N supply may exceed demand.

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CHAPTER 5

Summary and recommendations

5.1 Synopsis

In the dairy producing area of the southern Cape, grass pasture systems achieve high yields by maintaining high N fertilisation rates. In an attempt to reduce intensive use of N fertilisers and simultaneously increase pasture quality, legumes are incorporated into these grass pasture systems. Since legumes fix atmospheric N and thus provide an additional input of N, N fertiliser recommendations for grass pastures are not necessarily applicable to grass-legume mixed pastures. Literature states that inorganic N may negatively affect the production and persistence of legumes (Ledgard and Steele 1992; Ledgard 2001) Furthermore, research concerning the ideal N fertilisation regime for grass-legume pastures is limited, not only for South Africa, but also elsewhere in the world. Moreover, there is a paucity of such information for kikuyu-based pastures. This study was therefore conducted to determine the effect of different N fertilisation regimes [0 (N0), 20 (N20), 40 (N40), 60 (N60) kg N ha^{-1} and N_{var}] on soil and plant parameters for grass-legume pastures. This synopsis aims to answer each research question outlined in Chapter 1, and to identify limitations of the study.

How do N fertilisation rates influence soil N dynamics in a grass-legume pasture under grazing?

For this study, N inputs and losses were taken into account by the treatment referred to as N_{var}. For the N_{var} treatment, soil nitrate concentration was measured from wetting front detectors (WFDs) at depths of 150 and 300 mm. This method was not feasible in practice as water can only be collected from the WFD once soil water potential reaches values between -2 and -3 kPa. Irrigation was scheduled to maintain soil water potential between -10 and -25 kPa and under good irrigation management, deep percolation is prevented, therefore nitrate concentration could not be determined regularly. Furthermore, nitrate concentration measured from the 150 mm WFD was higher than the threshold value of 75 mg L^{-1} throughout most of the study. According to earlier suggestions by Fessehazion *et al.* (2011), N fertilisation is only necessary when the soil nitrate concentration is lower than 50 mg NO_3^- L^{-1}. Regardless, even following shifting this recommendation to a less strict concentration of 75 mg NO_3^- L^{-1}, N was only fertilised once on these plots for both the kikuyu-perennial ryegrass-clover (KPrCl) and kikuyu-lucerne (KL) pasture types. Nitrate concentration measured from the WFD planted at a 300 mm depth, varied minimally throughout the study, indicating that leaching was constant throughout the study for treatment N_{var}. Although nitrate was only measured for N_{var}, total soil N (TSN) distribution was determined for each treatment. The distribution of TSN between soil depths of N_{var} was similar to other treatments throughout the study, for both pasture types. This indicates that treatment probably did not have an

influence on N leaching during this study. Likewise, treatment had no effect on total soil N. Total soil N did, however, decrease from winter to spring for all treatments and for both pasture types. The decrease in TSN is most likely due to an increase in yield over the same time period. Even though TSN was not affect by N treatment, total inorganic soil N increased under the N60 fertilisation regime for both pasture types. This indicates that the soil became N saturated under N60 regime over time. Nitrogen fertilisation treatments for both pasture types had a minimal effect on potentially mineralisable N and urease activity throughout the study. Application of these two indicators for cultivated pasture studies on soils that have been built up with soil organic matter through time, as a result of minimum-tillage, are thus questionable, as results are not sensitive enough to show treatment effects.

Will N fertilisation rate affect herbage yield of a grass-legume pasture?

Herbage yield response to N fertilisation was poor. For the KPrCl pasture type, treatment had no effect on annual or seasonal herbage yield, except in the spring of year one, were treatment N60 produced a yield higher than N0 and N_{var}. For the KL pasture type, N fertilisation influenced the annual- as well as seasonal yield. Treatment N60 produced the highest or similar to the highest yield from spring to autumn in year one. Treatment N20, N40 and N60 produced equal annual yields, but were higher than N0 and N_{var}.

How will botanical composition of a grass-legume pasture be affected by N fertilisation rate?

Botanical composition for the KPrCl pasture type was influenced by N fertilisation rate. The kikuyu component was positively related to N fertilisation in the autumn. The ryegrass component was positively related to N fertilisation in the spring, summer and winter. The clover component was negatively correlated to N fertilisation throughout the trial period. The application of N may be advantageous for the kikuyu and ryegrass components in their respective active growing seasons, however N application will be to the detriment of the clover component. For the legume component to contribute to N fixation and pasture quality, it should be kept above the threshold value of 30%. Throughout the trial period, any application of N led to the clover component decreasing below this threshold value. When a clover component is incorporated into grass pastures to fix N or to improve pasture quality it is recommended that no N is applied.

For the KL pasture type, N fertilisation rate did not affect botanical composition of any species, except lucerne in the summer, during which the lucerne content of N40 was the highest. The high contribution of the weeds component of the KL pasture type's sward, may have limited the response to N fertilisation. The weeds component was more than 30% in the autumn of year one and two. Since lucerne and kikuyu are dormant during the winter and spring, other pasture species, such as weeds, dominated the sward during this time period. This may have affected nutrient uptake and growth of kikuyu and lucerne. Weeds were a limitation to this pasture type.

Will N fertilisation rates affect herbage crude protein?

The crude protein content of the KPrCl pasture type was the highest or similar to the highest under treatment N60 throughout the study. For the KL pasture type crude protein content was the highest or similar to the highest under treatment N60 from February to June.

5.2 General conclusion

Treatment N_{var} was used to determine a variable N application through the use of a WFD. This method was, however, unsuccessful. Nitrate concentration could not routinely be determined and limited data was available. Although this method was unsuccessful, soil as well as pasture parameters could be used to determine a variable N application rate.

Total soil N decreased from winter to summer for all treatments under both pasture types. This could be ascribed to an increase in yield in the coinciding period, and thus an increase in N uptake. A variable N application rate will therefore have to increase from winter to summer. Nitrogen treatment had a limited effect on most soil parameters except total inorganic soil N. Total inorganic soil N increased under the N60 treatment throughout the study for both pasture types. This is consistent with herbage crude protein content which was the highest or similar to the highest under treatment N60. These results indicate that the soil becomes N saturated under the N60 regime and that the fertilisation rate exceeds the pasture's N need. Over-fertilisation may thus occur under a N60 fertilisation regime in kikuyu-legume pastures. For both the KPrCl and KL pasture types, it is recommend that a variable N fertilisation rate, even during periods when pasture production rates are at their highest, should not exceed N60.

For the KPrCl pasture type, annual yield was not affected by the N fertilisation regime, however the clover component was negatively related to N fertilisation rates. The application of N decreased the clover component below the threshold value of 30%. The results of this study indicate that N0 may be sufficient for optimal pasture production if the aim is to maximise the clover component, however this is only the first year of the study. As the study continues N levels may be depleted. Studies conducted in New Zealand, on ryegrass-clover pastures, showed that an N application rate of 200 kg ha^{-1} yr^{-1} is ideal for pasture production (Clark and Harris 1996; Ledgard *et al.* 2001). For a KPrCl pasture, it is recommended that N fertilisation should be kept between N0 and N20 (Table 5.2.1), where treatment N20 is recommended for seasons with higher growth (spring and summer). Conventional N fertilisation guidelines propose that N has to be fertilised at a constant rate throughout the year, but the pasture's N requirement varies between seasons. The suggested variable N fertilisation rates therefore increase as pasture yield increases (Table 5.2.1). The suggested variable N fertilisation guidelines will potentially reduce N fertilisation by more than 50%. The main reduction is suggested for the winter and autumn.

For the KL pasture type, yield increased with N fertilisation treatment. The annual yield of treatment N20, N40 and N60 was higher than N0 and $N_{var.}$ Botanical composition was not influenced by N fertilisation regimes and therefore it is recommended that the N fertilisation rate for this pasture be maintained between N20 and N40. Treatment N20 may be applied in seasons characterised by lower yields (winter and spring), while N40 will have to be applied in the seasons with higher pasture yield potential (summer and autumn) (Table 5.2.2). Similar to the KPrCl pasture type, the suggested variable N fertilisation rates decrease as pasture yield decreases, therefore only half of the conventionally applied N is suggested for winter and autumn. Consequently, total annual N will decrease from 360 to 280 kg N ha^{-1} yr^{-1} if the suggested variable N regime is followed instead of the conventional fixed N application regime.

Table 5.2.1. Comparison between a conventional N fertilisation regime and a variable N fertilisation regime for a kikuyu-perennial ryegrass-clover pasture.

Season	Grazing cycles per season	Conventional N rates (applied after grazing) *	Total seasonal N (conventional system)	Suggested variable N rates (applied after grazing)	Total seasonal N
Winter	2	40	80	0	0
Spring	3	40	120	20	60
Summer	3	40	120	20	60
Autumn	1	40	40	0	0
Total annual N			360		120

*Beyers (1973)

Table 5.2.2. Comparison between a conventional N fertilisation regime and a variable N fertilisation regime for a kikuyu-lucerne pasture.

Season	Grazing cycles per season	Conventional N rates (applied after grazing) *	Total seasonal N (conventional system)	Suggested variable N rates (applied after grazing)	Total seasonal N
Winter	2	40	80	20	40
Spring	2	40	80	40	80
Summer	3	40	120	40	120
Autumn	2	40	80	20	40
Total annual N			360		280

*Beyers (1973)

5.3 Recommendations for further research

The use of a WFD to determine nitrate concentration of soil water was unsuccessful. For this method to be successful, irrigation scheduling will have to be adjusted. This is not practically feasible and will not be implemented by dairy producers. Other on-farm methods of determining soil N should be investigated in future studies. Furthermore, research concerning the cycle of N on farms is limited. Producers have limited knowledge on the N inputs and losses in their systems. A better understanding of the N cycle may assist producers in quantifying N flows within their systems and thus lead to better N management. Further research into these processes could lead

to enhanced understanding of the N cycle and therefore improve N fertilisation efficiency of cultivated pastures.

Throughout the study, all parameters showed a limited response to N fertilisation regimes. This may be ascribed to the effect of long-term intensive N fertilisation practices. The trial area was previously planted under minimum-tillage practices and received N fertilisation rates in excess of 300 kg N ha^{-1} yr^{-1} for at least 10 years. This likely resulted in the accumulation of soil N. Furthermore; N may have been carried over, through cow excreta, between experimental units. Since the trial area was strip-grazed, cows moved freely between experimental units. As result of these grazing management practices and the experimental design, excreta-N was theoretically distributed equally throughout the trial area. This might have reduced the effect of N treatments. Nitrogen treatments therefore had little effect on soil as well as pasture parameters. Additionally, both pasture types included a large invasive (grasses and broadleaf species not planted in trial area) species component. Although these species may contribute to yield, they compete for resources like sunlight, nutrients and water. These species may thus have contributed to the limited response to N fertilisation reported in the study. As a result weed control could have improved the yielding potential of planted species and may potentially have led to an enhanced response to N fertilisation. For the successful establishment of a kikuyu-lucerne pasture, weed control is therefore priority. Furthermore, it is recommended that lucerne is accompanied by pasture species that fill the gap in herbage production during the winter and spring. A pasture species such as a short duration Westerworlds type ryegrass could be suitable. Westerwolds ryegrass is an annual ryegrass that persists from establishment in autumn until spring (Van der Colf *et al*. 2015). This might decrease weed pressure in the period of winter and autumn and potentially increase the lucerne component as well as yield.

It is recommended that this study continues for at least five years. This will allow soil N levels to deplete and the effects of N fertilisation regimes may differ from results of this study.

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